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THE AGE ORDER OF EPIPHYSEAL UNION IN THE LONG BONES OF THE ALBINO RAT

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THREE PLATES (TWELVE FIGURES)

INTRODUCTION

Work on epiphyseal union in the skeleton of the albino rat has been carried on with the kind cooperation of Dr. H. H. Donaldson, The Wistar Institute. The time, order, and rate of the early ossification in this animal have already been comprehensively studied by Strong. The present paper, a brief report of which was read before the American Association of Anatomists last year (Dawson, '24), supplements Strong's findings by tracing the persistence of the cartilage plates and their final disappearance. Tables based on this report are included by Donaldson ('24). Since the preliminary report was made, many intermediate and some older stages have been studied, and in certain bones it has been possible to fix more definitely the time of union of the epiphyses. This paper is confined entirely to the long bones. Observations on the vertebral column and pectoral and pelvic girdles are not yet complete and will be made the subject of a later report. A brief discussion of the clavicle has already been published (Dawson, '25).

All material used in this study was obtained from the experimental colony at The Wistar Institute at different times during the past two years. With the exception of one old female, males only were used, as it seemed unwise to complicate the problem with the sex factor. Skeletons of all ages, from fourteen to 1270 days were examined. The one female

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studied had an age record of forty-seven to fifty months. The older rats were obtained through the kindness of Doctor Donaldson. The skeletons were fixed in either Gilson's fluid or 95 per cent alcohol. In decalcification nitric acid in 70 per cent alcohol or trichloracetic acid was used.

In the literature it is very difficult to find a complete account of the ossification history of any mammal, except man. Recently, Stevenson ('24) has completed a new survey of the sequence of human epiphyseal unions. He found that the sequence of epiphyseal union in man was definite and constant, and the epiphyses, according to him, may be divided into two classes: 1) the epiphyses of the so-called long bones which are characterized by a remarkable degree of constancy and, 2) the epiphyses of another group of bones, the innominate, scapula, ribs, and clavicle, which are subject to a certain degree of individual variation. Furthermore, Stevenson states that this same sequence of epiphyseal union is found quite generally throughout the majority of other mammalian forms and that it appears to be a remarkably constant and primitive mammalian character. The description of the material on which this generalization is based has not yet been published.

If this sequence of epiphyseal union is a primitive mammalian characteristic, it would seem possible that the same character might display itself in the more primitive vertebrates. Epiphyses are foreshadowed, but not developed in the Amphibians (Parsons, '05). In several groups of reptiles, especially the lizards, the epiphyses are present and highly developed (Dollo, '84; Parsons, '05; Moody, '07; Fuchs, '08). In birds, on the other hand, epiphyses rarely appear, although Fell ('25) claims that the long bones of the fowl possess structures homologous with the epiphyses of mammals. Accordingly, it is only in certain of the reptiles that it would be possible to follow the history of epiphyseal union. I have been unable, however, to find adequate data on the behavior of the epiphyses in reptiles to either substantiate or disprove the assumption that the sequence of epiphyseal union may be the same in reptiles as in mammals.

DESCRIPTION OF MATERIAL

a. The epiphyseal cartilage

In the actively growing bones the epiphyseal cartilages, so far as I have observed, are complete plates and are apparently not interrupted at any point. The cartilage cells usually show the typical arrangement in parallel columns. In older cartilaginous plates, that is, those which will soon be perforated by the invasion of osteogenetic tissue, the matrix of the cartilage usually appears definitely fibrillar. In certain areas, the cartilage cells and the homogeneous cementing substance soon disappear entirely (fig. 11) and the fibrillar substance which remains behind is easily seen. These fibrillar areas apparently undergo calcification and are then invaded by osteogenetic tissue (fig. 12). In this manner the cartilaginous plates become perforated by extensions of shaft marrow, and they are eventually broken up into more or less isolated masses of cartilage which are gradually removed and replaced by bone. The central area of the plate is ordinarily interrupted first. In most bones in the final stages of union of the epiphysis to the diaphysis, the epiphyseal plate is reduced to a narrow peripheral ring which is persistent in a few bones in the oldest skeletons examined.

b. The sequence and age of epiphyseal union

In the following description the various bones will not be considered individually, but their epiphyses will be discussed in the order in which they unite to their shafts.

1. Capitulum and trochlea of the humerus. A single well-defined center of ossification for the capitulum and trochlea is present at fifteen days. At thirty-one days, the cartilage plate is incomplete (fig. 1), and at forty-two days the epiphyseal cartilage has entirely disappeared. This is the first epiphysis of a long bone to unite with its shaft. The cartilage cells of the plate have not been observed to assume the characteristic arrangement in columns and the plate is usually broken through first at its periphery rather than at the center,

as is usually the case. The rate of union is so rapid that the typical sequence of events apparently is not followed.

2. The proximal epiphysis of the radius. The cartilaginous plate in this end of the radius is complete at fifty-eight days (fig. 2); but at sixty-nine days the plate gives evidence of being perforated (fig. 11). Two weeks later, portions of the epiphyseal cartilage are still present, and at ninety-two days bony union is complete.

3. The distal epiphysis of the tibia and the tuberosity of the calcaneus. At the age of sixty-nine days the epiphyseal cartilage is complete (fig. 3); two weeks later, this cartilaginous plate is interrupted by osteogenetic tissue, and at ninety-two days the cartilage has almost disappeared, but traces of cartilage can be seen as late as ninety-eight days. At 130 days, although no cartilage is present, the line of union is still indicated by the arrangement of trabeculae, but at 236 days there is no evidence of an epiphyseal line, and there is nothing in the arrangement of the trabeculae to show where the union of the elements had occurred. The tuberosity of the calcaneus, in its ossification history, parallels closely the distal epiphysis of the tibia, and is mentioned because it is always seen in sagittal sections of this region.

4. Distal epiphysis of the fibula. The ossification history of the lower end of the fibula is somewhat complicated by the intimate union which occurs between this part of the bone and the adjoining tibia. In general the ossification of the fibula follows closely that of the tibia, but it always appears to lag slightly. At ninety-eight days, when the distal epiphyseal cartilage of the tibia is present only as small isolated masses, the cartilage plate in the fibula is usually complete (fig. 4). At 130 days the cartilage is still present, but is greatly reduced and frequently perforated by invading osteogenetic tissue.

5. The medial epicondyle of the humerus. The ossification center for this bony process remains separate much longer than the epiphysis of the capitulum and trochlea. At ninety-eight days epiphyseal cartilage is complete. At 130 days

the cartilage plate has an appearance much like that of the proximal end of the radius at sixty-nine days, and complete union does not occur until four weeks later.

6. The olecranon process of the ulna. There is quite a long time interval between the union of the first five epiphyses described and the union of the remaining ones. At 401 days the cartilage plate in the olecranon process is distinct and prominent. At 790 days there is no evidence of any interruption of the plate and at 940 days remnants of the epiphyseal cartilage are still present.

7. Femur head and greater trochanter. At 940 days the cartilage plate shows several fibrillar areas which appear before perforation of the plate occurs. At 1006 days the cartilaginous plate is interrupted at several points (figs. 5 and 12), and isolated masses of cartilage are present as late as 1091 days. At 1135 days no cartilage can be found, and all traces of the epiphyseal line have been obliterated, but in the old female, forty-seven to fifty months of age, the cartilage plate was still present. The greater trochanter has a similar ossification history and does not merit a separate description.

8. The distal end of the femur and proximal end of the tibia. These epiphyses will be considered together, since the material at my disposal did not show any differences in their ossification. At 940 days the cartilage plates were continuous, but showed fibrillar areas. At 1006 days (figs. 9 and 10) the invasion of the cartilage had proceeded but slightly, but at 1135 days and at 1270 days only a peripheral ring of cartilage remained. In the one old female rat examined (age forty-seven to fifty months) the cartilage plates were still present and presented a picture similar to that of a male—aged 1006 days.

9. The proximal end of the fibula. The union of the proximal epiphysis like the distal epiphyseal lags behind that of the corresponding parts of the tibia. Figure 6 shows the condition at 1135 days. At this age the cartilage plate in the tibia is reduced to an inconspicuous peripheral ring. The delayed union of the epiphysis of the fibula may explain the decided bowing of this bone from the straighter tibia.

10. The distal ends of the radius and ulna. So far as could be determined from a study of the material, there is little choice between the distal ends of the radius and ulna and the head of the humerus, so far as rate of epiphyseal union is concerned. The oldest radius and ulna examined (1135 days) showed quite prominent plates with but few interruptions (fig. 8). A comparison of figure 8 with figure 7 will show the epiphyseal cartilage of the humerus to be slightly more cellular, probably indicating a slower rate of union.

11. The head of the humerus. This is apparently the last epiphysis of the long bones to become united to its shaft. As noted above, the line of cartilage is practically complete at 1135 days (fig. 7). At the age of 1270 days only a peripheral ring of cartilage remains. In the skeleton of the old female (forty-seven to fifty months) the head of the humerus, however, showed a stage comparable to that of the male 1006 days old.

The following is a summarized comparison of the order and age of epiphyseal union of the long bones of man and the albino rat:

Man (Stevenson, '24)

1. Distal extremity of humerus, 14th to 15th year.
2. Medial epicondyle of humerus, 16th year.
3. Olecranon of ulna, 16th to 17th year.
4. Head of radius, early 18th year.
5. Head of femur, early 18th year.
6. Lesser and greater trochanters of femur, early 18th year.
7. Distal extremities of tibia and fibula, 18th year.
8. Proximal extremity of tibia, 19th year.
9. Proximal extremity of fibula, 19th year.
10. Distal extremity of femur, 19th year.
11. Distal extremities of radius and ulna, 19th year.
12. Head of humerus, 20th year.

Albino rat

1. Distal extremity of humerus, 31–42 days.
2. Head of radius, 83–92 days.
3. Distal extremity of tibia, 92–98 days.
4. Distal extremity of fibula, 98–130 days.
5. Medial epicondyle of humerus, 130–158 days.
6. Olecranon of the ulna, 940 days.

7. Head of femur, 1006–1091 days.
8. Great trochanter, 1006–1091 days.
9. Distal end of femur, 1135 days.
10. Proximal end of tibia, 1135 days.
11. Proximal end of fibula, 1135+ days.
12. Distal extremities of radius and ulna, 1135+ days.
13. Head of humerus, 1135–1270 days.

DISCUSSION

Perhaps the most striking feature of the ossification history of the long bones of the rat is the remarkable persistence in certain bones of the plates of epiphyseal cartilage, remnants of cartilage being present in the oldest male (1270 days) examined. This persistence of epiphyseal cartilage appears to be in harmony with the long-continued skeletal growth as determined by direct measurements (Donaldson, '24). The data on the growth of very old rats, however, are very meager, and no accurate correlation can be made between the histological appearance of the epiphyseal cartilage and its relation to the process of growth. Stevenson ('24) takes exception to the universal acceptance of the view that the union of epiphyses and cessation of growth are interdependent phenomena. He cites several examples where cessation of growth has not waited until the union of the epiphyses, and concludes that the phenomenon of epiphyseal union is essentially one of differentiation and should not be confused with the process of growth which manifests itself at the epiphyseal line.

In man epiphyseal union in the long bones is limited to a rather definite age period, fourteen to twenty years. In the rat, on the other hand, there appear to be two rather well-defined age periods in which epiphyseal union occurs. The first period is from birth to the end of the fifth month, the second, from the thirtieth month to senility. Practically all cartilage has disappeared from the epiphyseal lines of the long bones at the age of 1270 days, and a rat is considered old at three years. In the last few years rats in the Wistar Colony have attained ages considerably above three years, one female examined by the author being at least forty-seven

months old. In the relatively short life-span of the rat, therefore, there is an interval of practically two years in which differentiation of the persisting epiphyseal lines proceeds very slowly.

In man, Stevenson ('24) has found that the sequence of union among the various epiphyses is definite and constant. According to him, this same sequence is found quite generally throughout the majority of other mammalian forms. In general, the same sequence is observed in the rat as in man, but there are several well-marked exceptions. The union of the median epicondyle of the humerus occurs relatively late in the rat. The union of the epiphyses of the distal ends of the tibia and fibula in the rat precedes that of the head and great trochanter of the femur, while the reverse is true in man. The union of the epiphyses of the olecranon process is also delayed in the rat. Of the three exceptions noted, the late union of the epiphysis of the head of the femur is the most important. This epiphysis, according to Parsons' classification, is a pressure epiphysis, while the epiphyses of the olecranon of the ulna and medial epicondyle of the humerus are traction epiphyses. The behavior of pressure epiphyses is believed to be more constant than that of traction epiphyses. In the rat there can be no question of the relatively late union of the epiphysis of the femur head, since it comes in the second age period of epiphyseal union, i.e., after the thirtieth month. Stevenson finds that this epiphysis in man is united before those of the distal extremities of the tibia and fibula which in the rat unite early—about 130 days of age.

Cope ('20) has recently published some observations on the persistence of fusion lines in the bones of the human skeleton. In man, for the most part, the ends of the long bones which exhibit a persistence of the fusion lines are the same ends whose epiphyses in the rat do not unite until the second age period of union. Whether this is of any fundamental significance or merely a coincidence it is difficult to say.

As stated in the introduction, male rats were used as a basis for this study. Strong ('25) has not noted any differ-

ence in the time or rate of ossification in the two sexes of the rat. Stevenson ('24) has also failed to note any sex difference in the time of union of the various epiphyses in man. This is at variance with the generally accepted view that the epiphyses in the female unite somewhat earlier than in the male (Pryor, '23). In my survey of rat material only one old female was studied, but in this animal all the epiphyses which unite in the second age period were still separate, while in the oldest male studied, 1270 days old, union of all epiphyses of the long bones was practically complete. This isolated case may be only a striking individual variation and not have any relation whatever to sex. This must remain an open question, however, until a sufficient number of skeletons of old female rats has been obtained for study.

SUMMARY

The sequence of union of the various epiphyses of the long bones in the albino rat is definite and constant. Although epiphyseal union as a whole may show some individual variation, that is, may be retarded or accelerated, still the order is always the same.

The epiphyses may be divided into two groups according to the age period in which they unite to their respective shafts. The age periods are as follows: 1) from first month to the end of the fifth month and, 2) from the thirtieth month to senility. The epiphyses of group 1, which unite in the first age period, are as follows: distal extremity of the humerus, head of the radius, distal extremity of the tibia, distal extremity of the fibula, and the medial epicondyle of the humerus. The epiphyses of group 2, which unite late in life, are as follows: olecranon of the ulna, head of the femur, greater trochanter, distal end of the femur, proximal end of the tibia, proximal end of the fibula, distal extremities of the radius and ulna, and the head of the humerus.

The sequence of epiphyseal union in the rat parallels in general the order of union found in man. Three exceptions are noted: the medial epicondyle of the humerus, the olecranon

process of the ulna, and the head and great trochanter of the femur. All unite relatively late in the rat.

Present records do not show just how long the growth of the skeleton continues in the rat. The histological evidence presented here indicates that until old age, growth is possible in at least one end of all the bones found in the proximal and distal segments of both the fore and hind limbs.

The data given in this paper are based on a study of the skeletons of male rats. The influence of sex has not been critically studied.

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PLATES

Figures 1 to 10 were photographed at the same magnification (32-mm. objective) and figures 11 and 12 at a greater magnification (16-mm. objective).

PLATE 1

EXPLANATION OF FIGURES

1 Sagittal section of the elbow of a rat thirty-one days old, showing the trochlea of the humerus and the olecranon process of the ulna. Note the incomplete cartilage plate in the humerus at *a* and the epiphyseal center developing in the olecranon at *b*.

2 Sagittal section of the elbow of a rat fifty-eight days old, showing the trochlea of the humerus and the head of the radius. Note the disappearance of the cartilage plate in the humerus and the prominent plate in radius at *c*. Compare this with figure 11 at the age of sixty-nine days when the cartilage plate of the radius first shows signs of perforation.

3 Sagittal section of the ankle of a rat sixty-nine days old, showing the distal end of the tibia and the talus. At this age the cartilage plate is complete and active (*d*). At ninety-eight days the plate is obliterated. Compare with the fibula (fig. 4).

4 Sagittal section of the distal end of the fibula of a rat ninety-eight days old. The plate is still complete, but is very thin (*e*).

5 Sagittal section of the head of the femur of a rat 1006 days old. Cartilage plate (*f*) is present, but is being perforated in many places. See figure 12, under a higher magnification.

c
2
e
4
f
5
d
by Madge Walsh
3
a
b
1

PLATE 2

EXPLANATION OF FIGURES

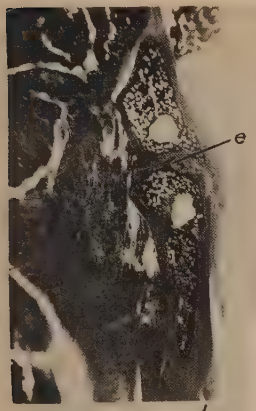
6 Frontal section of the proximal end of fibula in a rat 1135 days old. Cartilage still present (*e*), perforated in several places and very thin. Compare with the distal end (fig. 4), age ninety-eight days.

7 Sagittal section through the head of the humerus of a rat 1135 days old, cartilage plate present (*b*), reduced in thickness and perforated occasionally by developing bone.

8 Frontal section of the wrist of a rat 1135 days old, showing the distal ends of radius (*c*) and ulna (*d*). Cartilage plates in much the same condition as in the head of the humerus (fig. 7).

9 Sagittal section of the distal end of the femur of a rat 1006 days old. Cartilage plate still complete and quite thick (*f*).

10 Sagittal section of the proximal end of the tibia of a rat 1006 days old. Cartilage (*g*) in much the same condition as that of the distal end of femur of the same age (fig. 9). Note the ossification of the meniscus at *a*.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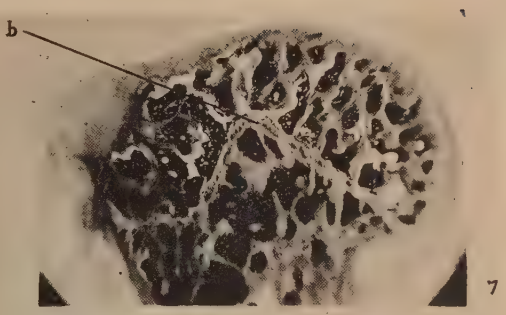
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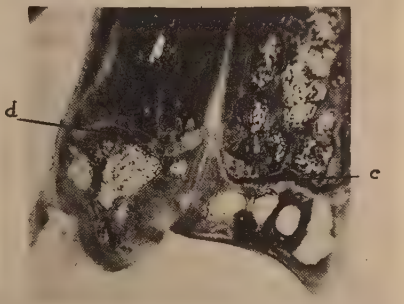
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f
by Madge Walsh
9

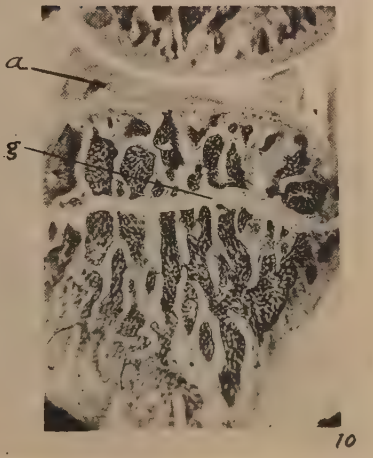
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PLATE 3

EXPLANATION OF FIGURES

11 Cartilage plate at the proximal end of the radius of a rat sixty-nine days old. Epiphysis above. Note the large number of cartilage cells present. The plate is being perforated at *a*. Note the fibrillae in this region.

12 Cartilage plate at the proximal end of the femur in a rat 1006 days old. Epiphysis above. Note the number of cartilage cells present. Compare with figure 11. The plate is the same thickness as in figure 11 and is being perforated at *b* and *c*. Note the calcified fibrillae at *c* and the newly formed bone at *d*.

a
11

b
d
c
by Madge Walsh.
12

THE EFFECT OF VASECTOMY ON SWINE[1]

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Many workers have studied the effects of vasectomy on several different species of animals, including man. The extravagant claims made by Steinach ('20) revived interest in both the clinical and biologic phases of the problem. He maintained that vasectomy, or ligation of the vas deferens, always leads to degeneration of the germinal epithelium and hyperplasia of the interstitial cells. The increase in the number of interstitial cells was supposed to produce more testicular hormone, and so stimulate rejuvenation. Moore ('23), Moore and Oslund ('24), and Oslund ('24) have shown that vasectomy of itself does not cause degeneration of the seminiferous tissue. Their work shows that when degeneration does take place, it is due to other causes, usually to artificial cryptorchidism. Adhesions subsequent to operation may cause the retention of the testicles in animals having open inguinal canals. In recent papers Oslund ('24 a, b, c, d) has carefully reviewed the literature and pointed out that in several species of animals having closed inguinal canals no testicular changes followed vasectomy. Moore and Quick ('24) have explained the changes following artificial cryptorchidism as due to differences in temperature.

In connection with vasectomy on swine, the writer ('24) has made histologic sections of the testicles of three boars after operation. As a study of the literature has failed to reveal any records on swine, these findings are being re-

[1] Paper no. 52 from the Departments of Genetics and Veterinary Science, Wisconsin Agricultural Experiment Station. Published with the approval of the Director of the Station.

ported as a slight addition to the body of data from other species having closed inguinal canals.

Boar I. This was a pig 115 days of age when operated upon. Each vas deferens was ligated and ½ inch of the vas was excised. Mutilation of some of the adjacent structures led to atrophy of each testicle. Bilateral castration was performed 125 days after vasectomy. At this time the left testicle was only the size of a hazelnut and the right about the size of a hickory nut. The tissue was fixed with Allen's B-15, embedded in paraffin, and stained with Heidenhain's iron hemotoxylin. Sperm heads were numerous in the tubules. Notwithstanding the marked atrophy of each testicle, the histologic picture did not differ materially from that of normal testicular tissue.

Boar II. This boar was 213 days of age when operated upon. A piece of the vas deferens of each side was removed after ligation in two places. Smears were made from the excised pieces of each vas deferens and spermatozoa demonstrated by microscopic examination. No atrophy or other unfavorable sequelae followed the operation. Bilateral castration was performed 126 days after vasectomy. The epididymis of each testicle was found to be enormously distended with spermatic fluid up to the point of occlusion. On puncturing the epididymis, a miniature geyser of spermatic fluid resulted. A drop of this was mixed with normal saline solution, warmed, and examined microscopically. Very numerous motile spermatozoa were observed. Pieces of testicular tissue were fixed in Flemming's strong solution, embedded in paraffin, and stained with Heidenhain's iron hemotoxylin. The histologic picture appeared normal.

Boar III. A boar one and one-half years of age was operated upon in the same way as with boar II. No atrophy or other unfavorable sequelae ensued. The boar was killed and his testicles removed for study 393 days, or about 13 months, after vasectomy. The testicles appeared normal in every way. The vas was occluded in each case, yet there was no marked distention of the epididymis. Puncture of

the epididymis revealed copious spermatic fluid. Microscopic examination of a saline dilution of this demonstrated numerous motile spermatozoa. Pieces were fixed in Allen's B-15, Flemming's strong and Zenker's solutions, embedded in paraffin, and stained in the usual way. The histologic picture was normal.

About a week after vasectomy on boar III, this boar showed increased restlessness and sexual desire. The caretaker, who did not know the nature of the operation, remarked about the increased 'ranting' of this boar. After a few days this passed away and the boar acted perfectly normally. He was allowed to serve several sows after vasectomy, and the ejaculated fluid was examined microscopically. This was devoid of spermatozoa. To all appearances, his actions were the same as those of any normal boar. The other two vasectomized boars showed no noticeable changes in behavior after vasectomy.

The histologic findings reported above are identical with those reported from the other species of animals which have closed inguinal canals, as is the case in swine. They provide further evidence in support of Oslund's conclusion that vasectomy of itself does not cause degeneration of the seminiferous tissues.

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A CASE OF PERSISTENT LEFT SUPERIOR VENA CAVA WITH REVERSED AZYGOS SYSTEM

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TWO FIGURES

The subject is an adult male, whose clinical history was not obtainable. This is the second case of left superior vena cava observed in this laboratory in a series of 385 cadavers, principally adult males past middle life. The previous case was described by Huffmire and Bower ('19).

The heart is normal in size and development. The coronary sinus, however, is greatly dilated, with a large ostium joining it to the right atrium. The valves of the sinus are small and ineffectual, which probably accounts in part for the dilatation. The sinus measures 2 cm. in its greatest diameter, narrowing at both ends.

The right superior vena cava originates by the junction of the subclavian and internal jugular veins of that side. Its relations in the thorax and its termination in the upper part of the right atrium are normal. A slight bulge and fibrous remnant on its dorsal aspect, at the level of the upper margin of the pulmonary artery, marks the junction of the post-cardinal vein with the duct of Cuvier during development and would probably mark the site of the termination of a normal azygos vein.

The left superior vena cava originates by the junction of the left subclavian and internal jugular veins. Passing down into the thorax it lies ventrolateral to the left subclavian artery, and, just lateral to the origin of this vessel, it passes ventral to the arch of the aorta. Arching anterior to the

left branch of the pulmonary artery and superior and inferior left pulmonary veins, with a slight proclivity backward and to the right, it becomes continuous with the dilated coronary sinus.

The left innominate vein is represented by a vessel 2 mm. in diameter extending between the right and left superior venae cavae. Its attachment to the left superior vena cava is

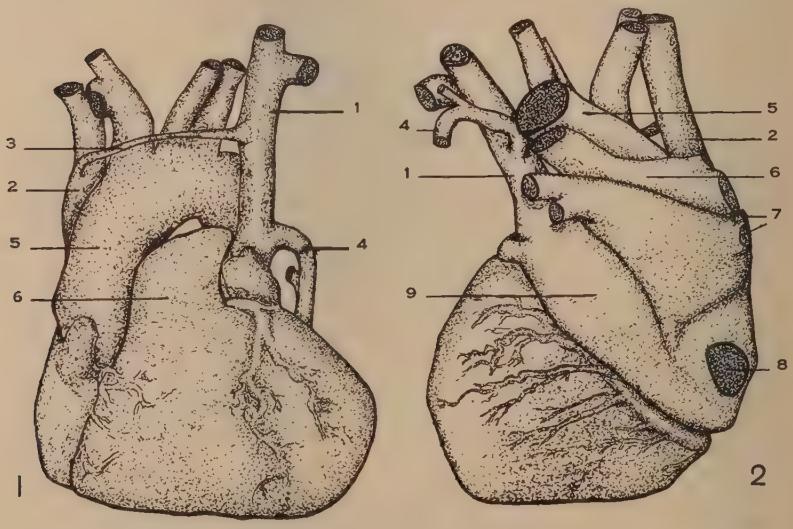

Fig. 1 Ventral view of heart and great vessels.

Fig. 2 Dorsal view of heart and great vessels.

1, left superior vena cava; *2*, right superior vena cava; *3*, anterior cardinal anastomosis; *4*, azygos vein; *5*, aorta; *6*, pulmonary artery; *7*, right pulmonary veins; *8*, inferior vena cava; *9*, coronary sinus.

2 cm. below the origin of that vessel, while on the right this distance is 4 cm.

The azygos system shows a true reversal, the hemiazygos and accessory hemiazygos draining the right side, the azygos draining the left. The hemiazygos has no connection with the right superior vena cava, a fibrous remnant on the latter (vide supra) probably marking the obliterated cephalic end of the right postcardinal vein. The hemiazygos, after re-

ceiving the accessory hemiazygos, crosses the middle line at the level of the eleventh thoracic vertebra and joins with the azygos. The azygos, passing up along the vertebral column and arching above the root of the left lung, joins at nearly right angles the posterior aspect of the left superior vena cava.

The occurrence of the reversed azygos system with a persistent left superior vena cava seems to be unusual. McCotter ('16) describes in his first case of persistent left superior vena cava azygos veins entering both the right and the left superior vena cava.

In the case previously described from this laboratory (Huffmire and Bower, '19) both posterior cardinal veins had persisted, with no anastomosis. Each had formed an azygos vein, one emptying into the right superior vena cava and one into the left.

Cameron ('15) cites a case described in the catalog of the Royal College of Surgeons' Museum, specimen 569.1. In this case the left superior vena cava is persistent, the right absent. The azygos of course joins the left superior vena cava.

As an interpretation of the anomalies shown by our specimen, we might offer the following possibility. Early loss of the communication between the right posterior cardinal vein and the right duct of Cuvier would result in the retention of the left posterior cardinal vein and probably would tend to retain the importance of the left duct of Cuvier as a drainage channel for the blood from the thoracic wall. This path could be utilized by the blood from the left side of the head and neck and from the left upper extremity, thus rendering the anterior cardinal anastomosis (which normally forms most of the left innominate vein) superfluous, and explain its diminutive size in this specimen.

In conclusion, the writers wish to express their appreciation of the help received from Prof. Wayne J. Atwell and Dr. R. R. Humphrey in the preparation of this report.

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THE DEVELOPMENT OF THE NOSE IN THE SPERMOPHILE, WITH PARTICULAR REFERENCE TO THE PART PLAYED BY THE PRIMITIVE CHOANAE IN THE FORMATION OF THE NASAL CAVITY IN MAMMALS

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TWELVE FIGURES

INTRODUCTION

Despite the vast amount of work that has been done on the development of the nose, a review of the literature leaves us very decidedly in the dark with reference to certain very essential phases. It was with a view to clarifying these stages that the work on which this report is based was undertaken. Since the literature contains no complete account of the development of the nose in the spermophile, it seems worth while to report here the general embryology of this region in the mammal chosen for this investigation. A complete discussion, however, is beyond the scope of this paper. Neither is it possible to consider here the extensive literature on the subject. This seems the less necessary in view of the recent work of J. P. Schaeffer, in whose publications a full report is to be had. The appended bibliography is intended merely as a reference to the literature pertinent to the particular phases in which we were primarily interested—the relationship of the primitive choanae to the formation of the nasal cavity. No pretense at an analysis of the lateral and medial nasal walls will be made at this time, such observations as have been made indicating that they do not differ materially from those of other rodents.

MATERIAL AND METHODS

The material on which this report is based consists of a very complete collection of spermophile embryos (Spermophilus tridecemilineatus Mitchell). The embryos are sectioned at 10 and 20 μ and stained with hematoxylin and eosin. No record of the age is available, the stage being recorded in crown-rump length. This measurement was taken previous to fixation. Incidentally, note may be made of the fact that the stage of development of the individual embryo corresponds very closely to the recorded length—an advance of 0.5 mm. in length showing a corresponding advance in the formation of the organs. Representative stages were selected and reconstructed by the Born wax-plate method. The following pages include a description of the wax models of these stages together with observations and interpretations based on microscopic study and on a comparison of the stages.

The writer wishes to make grateful acknowledgment to Dr. H. J. Prentiss for helpful suggestions and criticisms during the progress of this study and to Dr. E. M. MacEwen who collected this series of embryos for the department.

DISCUSSION

The first indication of the formation of the olfactory apparatus is in the appearance of the nasal placodes at about the 3-mm. stage. These structures appear near the lateral margins of the nasofrontal process. Due to the growth outward of the surrounding tissue, the nasal area is depressed to form the nasal pits; the primitive nasal cavities are formed by the growth posteriorly of these pits, as well as by their passive deepening by the continued growth outward of the region surrounding the original nasal area. In its growth posteriorly, the floor of the primitive nasal cavity tends to parallel the roof of the primitive buccal cavity. Each nasal cavity, as it extends into the soft tissue of the nasofrontal process, delimits a lateral portion of that process from the

central mass. Thus the nasofrontal process comes to consist of three parts, the medial nasal process lying between the nasal cavities and the lateral nasal processes lateral to the nasal cavities.

In an embryo of 8 mm. (fig. 1) the nasal cavities are separated by the wide medial nasal process and bounded laterally by the lateral nasal processes. Extending inferiorly and slightly laterally from each anterior naris toward the primitive mouth is a distinct groove, the bucconasal groove, which is bounded medially by the inferolateral portion of the medial nasal process (globular process of His) and laterally by the junction of the lateral nasal and maxillary processes. Posteriorly the primitive nasal cavity becomes markedly narrowed in its transverse diameter, appearing in cross-section as a slit-like cavity. Just in front of its blind posterior extremity it extends inferiorly and slightly medially toward the primitive buccal cavity (fig. 2). On the roof of the primitive buccal cavity, directly opposite this buccalward extension of the primitive nasal cavity, is a corresponding depression nasalward. These two depressions indicate the point where the nasal and oral epithelium later abut to form the bucconasal membrane (Hochstetter). At this time, however, the two epithelial layers are separated by a considerable thickness of mesenchymal tissue. Just lateral to the depression in the roof of the mouth is a slight elevation on the maxillary process representing the beginning palate process. On the superior surface of the mandibular process the anterior anlagen of the tongue appear as two lateral elevations separated by a broad shallow groove.

Reference to a model of a 9-mm. embryo (fig. 3) shows the anterior nares still widely separated by the medial nasal process. Study of sections through the anterior nares at this time reveals a distinct thickening of their epithelial wall, due to a piling up of the superficial cells (fig. 4). The lumina of the anterior nares are, therefore, somewhat diminished in size, but are still patent. The bucconasal groove is still apparent, but is partially obliterated at its buccal end by the

fusion of the maxillary with the globular process, this fusion progressing from behind forward. The nasal cavity is encroached upon medially by the bulging into it of the septal wall containing the vomeronasal organ. Irregularities are apparent on the lateral wall, due to the development of the turbinals. Posteriorly, the nasal cavities, as in the 8-mm. stage, end blindly. Well in front of its blind extremity each cavity has now extended toward the primitive buccal cavity until, over a very limited area, the nasal and buccal epithelium are in contact. This small area of abutting nasal and buccal epithelium forms the bucconasal membrane. On the roof of the primitive mouth the position of this membrane is indicated by a much more distinct depression than in the previous stage. Lateral to this depression the palate process is now a distinct elevation on the maxillary process. The mandibular process shows no change except in the size of the tongue and the obliteration of the groove between the two anlagen.

The 10-mm. embryo (fig. 5) presents some very marked changes over the previous stage. The medial nasal process has decreased somewhat in thickness, so that the anterior nares are less widely separated. Although the anterior nares are distinctly patent, the lumina are markedly decreased in size as a result of the further piling up of the surface epithelium of their walls. The bucconasal groove has been obliterated by the fusion of the maxillary and lateral nasal processes with the medial nasal process. The bucconasal membrane has now ruptured, and communication has been established between the primitive nasal and buccal cavities. Lateral to the buccal openings of the primitive choanae are the palate processes which now extend from a point opposite the anterior margin of the primitive choanae well beyond their posterior limit. These processes present in the mouth cavity in the angle between the tongue and the floor of the mouth, the gingiva not yet having been differentiated. At this stage of development the nasal apparatus consists of the two primitive nasal cavities opening to the exterior through the anterior nares and communicating with the primitive buccal cavity

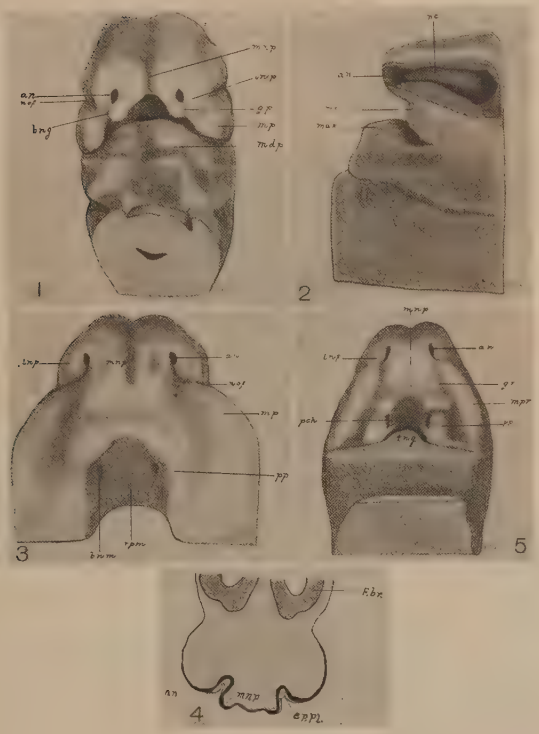

Fig. 1 Model of face region of an 8-mm. embryo. × 12.5. *an*, anterior naris; *nof*, naso-optic furrow; *bng.*, bucconasal groove; *mnp*, medial nasal process; *gp*, globular process; *lnp*, lateral nasal process; *mp*, maxillary process; *mdp*, mandibular process with tongue.

Fig. 2 Lateral view, head region of an 8-mm. embryo. × 12.5. *an*, anterior naris; *nc.* nasal cavity; *mp.*, maxillary process; *mdp*, mandibular process.

Fig. 3 Model of nose region and roof of mouth, 9-mm. embryo. × 12.5. *an*, anterior naris; *nof*, naso-optic furrow; *lnp*, lateral nasal process; *mnp*, medial nasal process; *pp*, palate process; *rpm*, roof primitive mouth; *bnm*, bucconasal membrane.

Fig. 4 Sketch of section through anterior nares, 9-mm. embryo. × 12.5. *an*, anterior naris; *ep.pl*, epithelium being piled up to form epithelial plug; *mnp*, medial nasal process; *F.br*, floor of forebrain.

Fig. 5 Model, roof of mouth and nose region seen from below, 10-mm. embryo. × 12.5. *mnp*, medial nasal process; *an*, anterior naris; *lnp*, lateral nasal process; *gr*, groove between maxillary and medial nasal processes; *mpr*, maxillary process; *pp*, palate process; *pch*, primitive choana; *tng*, tongue.

through the primitive choanae. The primitive nasal cavities are separated from each other by the thick primitive nasal septum and from the primitive buccal cavity in front of the primitive choanae by the primitive palate, a thick mass of mesodermal tissue. As will be seen from a study of this and later stages, the primitive nasal cavity, from the time of the formation of the primitive choanae until the definitive nasal and mouth cavities have been formed, always extends somewhat posteriorly beyond the posterior margin of the primitive choanae. Thus the primitive palate does not form the entire floor of the primitive nasal cavity, a portion of that floor lying behind the primitive choanae. The primitive choanae are formed between the lateral and medial nasal processes, these processes forming the roof of the primitive buccal cavity. The tongue is well developed, lying in the interval between the palate processes and extending only as far forward as the anterior margin of the primitive choanae.

In an embryo of 10.5 mm. (fig. 6), the reconstruction of which is featured here with the permission of Dr. E. M. MacEwen, the primitive choanae are seen to be established and the anterior nares closed by an epithelial plug formed by the proliferation of their lining epithelium. The nasal cavity is represented as a cast and the floor of this cavity is seen to parallel the roof of the primitive mouth. The position of the primitive choanae with reference to the buccal portion of the hypophysis is to be noted. This embryo represents a stage at the time of the establishment of the primitive choanae and these openings are seen to be, at this time, nearer the anterior nares than to the buccal portion of the hypophysis.

The 14-mm. embryo (fig. 7) is chosen as representing a decided advance in the development of the nasal and mouth structures. The anterior nares remain completely closed by their epithelial plugs, their position being indicated externally by a marked depression of the plugs. That portion of the nasal cavity lying immediately posterior to the plug is a transversely narrowed slit; its roof is somewhat lower and its floor somewhat higher than the roof and floor of the cavity

proper. Posteriorly, the contour of the cavity conforms in general to that of the earlier stages, the walls presenting more marked irregularities due to advance in development of the turbinals, etc. The primitive choanae are now mark-

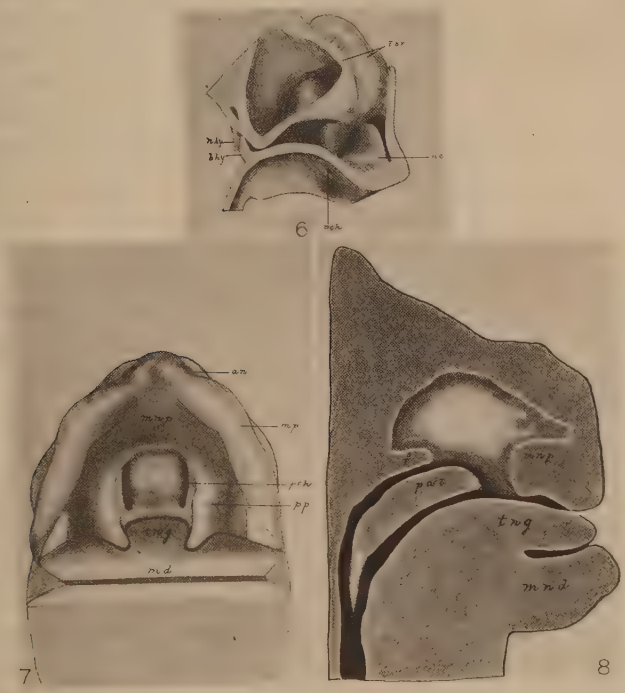

Fig. 6 Sagittal section of model of nose and brain region of an embryo of 10.5 mm. × 12.5. *nc*, nasal cavity represented as a cast; *Fbr*, forebrain; *pch*, primitive choana; *nhy* and *bhy*, neural and buccal portions of hypophysis. (Reconstruction by Dr. E. M. MacEwen.)

Fig. 7 Inferior view of roof of mouth and nose region. Model of a 14-mm. embryo. × 12.5. *an*, anterior naris; *mnp*, medial nasal process; *mp*, maxillary process; *pch*, primitive choana; *pp*, palate process; *tng* and *md*, tongue and mandible in cross-section.

Fig. 8 Model of septal wall of a 16-mm. embryo. × 12.5. *mnp*, medial nasal process forming anterior boundary of primitive choana and of canal of Stenson; *f*, floor of primitive nasal cavity forming posterior boundary of primitive choana; *pal*, sagittal section of definitive palate which forms posterior boundary of canal of Stenson; *tng*, tongue; *mnd*, mandible.

edly elongated anteroposteriorly as a result of the gradual recession of the floor of the primitive nasal cavity posterior to the choanal apertures. While the posterior margins of the choanae are carried posteriorly by this recession, there is apparently little, if any, extent anteriorly of the anterior margins of the choanae. This latter fact is evident in that the anterior margins of the choanae remain approximately opposite the anterior margins of the palate processes from the time of their first appearance. The primitive palate, however, grows anteriorly together with the other structures, so that the distance between the anterior margins of the choanae and the anterior nares is increased. Thus the migration posteriorly of the primitive choanae spoken of in the literature (J. P. Schaeffer) is really a recession of the floor of the primitive nasal cavity (posterior margin of primitive choanae) plus a growth anteriorly of the nose and mouth structures in the development of the face. The palate processes now extend well lingually in the angle between the tongue and the inferior gingiva, occupying practically the entire primitive buccal (bucconasal) cavity between the tongue and gingiva. The tongue is now a well-developed organ occupying that part of the primitive buccal cavity between the palate processes and extending well forward in front of the anterior margin of the primitive choanae.

A 16-mm. embryo (fig. 8) shows marked enlargement of the anteroposterior and vertical diameters of the primitive nasal cavities, while the transverse diameter remains relatively slight. The anterior nares are still closed by the epithelial plugs. That portion of the cavity immediately posterior to the plug is very small, as compared to the more posterior portion, in both its vertical and transverse diameters. The primitive choanae are increased in their anteroposterior diameters, due to further recession of the floor of the primitive nasal cavities. The palate processes have now rotated medially from their lateral lingual position and have come in contact with each other and begun to fuse in the midline above the tongue. This fusion begins a short

distance behind the anterior margin of the primitive choanae and progresses posteriorly. Thus the anterior margins of the palate processes are free in this stage, forming the posterior boundary of a communication between the nasal and mouth cavities. This communication is the canal of Stenson, the anterior boundary of which is the medial nasal process at its juncture in this region with the lateral nasal process. The anterior margin of the primitive choanae becomes, therefore, the anterior boundary of the canal of Stenson, and the canal of Stenson may be designated as that portion of the primitive choana in front of the fusing palate processes. Fusion of the primitive nasal septum with the palate processes has not begun at this time. With the rotation and fusion of the palate processes just described, a portion of the primitive buccal (bucconasal) cavity is incorporated into the nasal cavity. This portion of the nasal cavity derived from the primitive buccal cavity is, however, a small portion, in so far as its vertical diameter is concerned; a larger portion of the primitive buccal cavity is occupied by the palate processes themselves. With the union of the palate processes we may speak of a definitive buccal cavity and a definitive nasal cavity. The latter is composed of the two primitive nasal cavities and the portion derived from the primitive buccal cavity. Each primitive choana now communicates above with its corresponding primitive nasal cavity. Inferiorly, each choana communicates in front of the fusing palate processes (definitive palate) with the definitive buccal cavity, and behind the anterior margin of the definitive palate with that portion of the definitive nasal cavity incorporated from the primitive buccal cavity as described above. The definitive nasal cavity posterior to the primitive choanae is now seen to consist of two portions, a superior portion derived from the primitive nasal cavity and an inferior portion derived from the primitive buccal cavity. These two portions are separated by that portion of the floor of the primitive nasal cavity not yet obliterated by the posterior migration of the posterior margin of the primitive choana. The two primitive nasal cavities are

separated by the primitive septum and, since the septum has not yet fused with the palate, the two primitive cavities communicate through the buccal derivative of the nasal cavity by way of their primitive choanae. Coincident with the rotation of the palate processes, there is a depression of the floor of the mouth which permits the tongue to free itself from its position between the palate processes and take a position inferior to them. In taking this position the tongue decreases in its vertical diameter and spreads laterally to occupy that portion of the buccal cavity formerly occupied by the lingually placed palate processes. The mechanism by which the tongue is freed from its position between the palate processes has received considerable attention in the literature and a number of theories have been advanced in explanation (Keibel: Human Embryology—Keibel and Mall). In observing the various stages the writer has been impressed with two factors which it seems must influence this change and will largely explain it. First, the general depression of the floor of the mouth tends to pull the tongue from between the palate processes. Second, the growth vertically of the maxillary process keeps pace with the general vertical growth and tends to lift the palate processes over the tongue.

In an embryo of 16.5 mm. certain noteworthy advances are apparent (fig. 9). The anterior nares remain closed by the epithelial plugs and the nasal and mouth cavities, which may now be spoken of as the definitive nasal and mouth cavities, still communicate in front of the definitive palate through the canal of Stenson. The primitive nasal septum has grown inferiorly and, just posterior to the canal of Stenson, has begun to fuse with the definitive palate in the midline. With the fusion of the septum and palate the canal of Stenson becomes a Y-shaped canal, the inferior limb of the Y opening into the definitive buccal cavity and each superior limb into its respective nasal cavity, the two nasal cavities being now partly separated by the beginning fusion of the septum with the palate. The lateral portions of the palate processes have now extended forward to fuse with the medial nasal process

at its juncture with the lateral nasal process and thus come to form the lateral limits of the canal of Stenson, decreasing its diameter. Posterior to the canal of Stenson, that portion

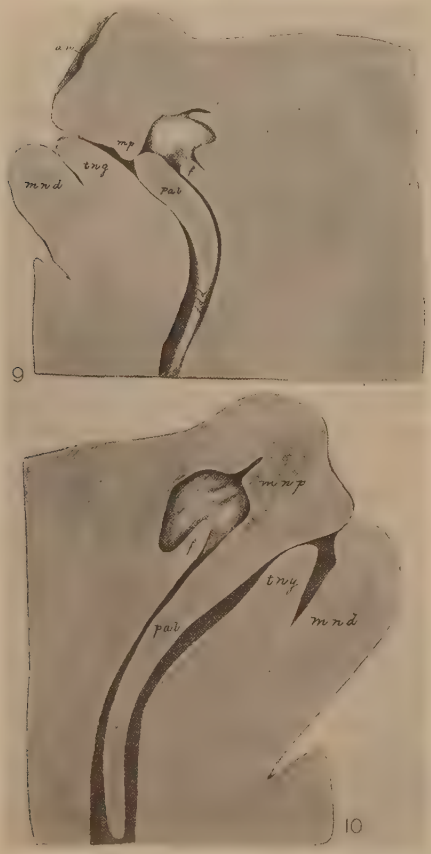

Fig. 9 Model of nose and mouth region of a 16.5-mm. embryo in sagittal section, septal view. × 12.5. *an*, anterior naris; *mp*, medial nasal process; *f*, floor of primitive nasal cavity; *pal*, palate process; *tng*, tongue; *mnd*, mandible; *ppf*, palatopharyngeal fold. Between *mp* and *pal* is the canal of Stenson, which is almost closed.

Fig. 10 Sagittal section through the nose region of a model of a 19-mm. embryo. × 12.5. *mnp*, medial nasal process; *f*, floor of primitive nasal cavity; *pal*, palate; *tng*, tongue; *mnd*, mandible.

of the definitive nasal cavity derived from the primitive nasal cavities and that derived from the primitive mouth cavity still communicate through the posterior part of the primitive choanae. Posterior to the primitive choanae the primitive nasal and buccal portions of the nasal cavity are separated, as in the previous stage, by the receding floor of the primitive nasal cavity, in which, at this time, cartilage has appeared.

The 19-mm. embryo (fig. 10) shows some very important changes in the parts of particular interest. The palate processes have fused throughout their entire extent. Fusion between the anterior part of the definitive palate and the medial nasal process (anterior margins of the primitive choanae) has also occurred, with the consequent obliteration of the canal of Stenson, in so far as the soft tissues are concerned. Bony foramina remain in this region between the maxillary and premaxillary portions of the hard palate. They are the foramina of Stenson and Scarpa and transmit the anterior palatine vessels and nerves. The canal of Stenson is completely closed in all embryos examined at this stage. The posterior margins of the primitive choanae remain practically as in the previous stage. Between the point of closure of the canal of Stenson and this posterior margin the primitive choanae remain as elongated slit-like communications between the primitive nasal and primitive buccal portions of the definitive nasal cavities. The fusion of the septum and palate is now practically complete, so that the two nasal cavities are separate. The line of closure of the canal of Stenson contains remnants of epithelium at this time (fig. 11, B). A definite depression is present on the nasal side at the upper limit of this line of fusion. The anterior nares are still closed by the epithelial plugs, in which, however, evidence of degeneration may be seen in the marked depression of the plug externally and, beneath this depression, a large area of liquefaction (fig. 11, A). In the floor of the primitive nasal cavity, which remains between the primitive nasal and buccal portions of the definitive nasal cavity and forms the posterior boundary of the primitive choana, the cartilage of the carti-

laginous nasal capsule has formed a definite transverse lamina extending from the cartilaginous septum to the lateral cartilage (fig. 11, B). This cartilaginous lamina has apparently

Fig. 11 Sketches through the nose region of a 19-mm. embryo. Above, through anterior naris. Below, near septum. × 12.5. *nc*, nasal cavity; *liq*, area of liquefaction in epithelial plug; *ep.pl*, epithelial plug in anterior naris; *sc*, septal cartilage; *f*, floor of primitive nasal cavity; *c*, cartilage extending into floor of primitive nasal cavity; *mxp*, bone in maxillary portion of palate; *pmxp*, bone in premaxillary portion of palate; *ep*, epithelial remnant at point of closure of canal of Stenson.

Fig. 12 Sketch of sagittal section through right nasal cavity of a 25-mm. embryo. × 12.5. *c,c,c*, portions of cartilaginous nasal capsule; *t,t,t*, turbinates; *f*, floor of primitive nasal cavity forming a transverse septum between this portion of the definitive nasal cavity and that portion derived from the primitive buccal cavity; *bc*, basilar plate of cartilage; *fbr*, floor of forebrain; *hy*, buccal portion of hypophysis; *pmxp*, bone of premaxillary portion of palate; *mxp*, bone of maxillary portion of palate; *hp*, horizontal portion of palate bone.

retarded, for the time being, the recession of the posterior margin of the primitive choana, the recesion having continued steadily until the appearance of this cartilage in about the 14-mm. stage.

Figure 12 represents a sagittal section through the right nasal cavity of a 25-mm. embryo and is presented here to call attention to certain features at this relatively late stage. It will be seen that the canal of Stenson is entirely obliterated and that no epithelium is now present in its line of closure. The anterior nares are again becoming patent, due to the degeneration of the epithelial plugs with which they were formerly filled. The cartilaginous nasal capsule is shown in its relation to the nasal wall. The floor of the primitive nasal cavity posterior to the primitive choana is still present and contains the well-formed lamina of cartilage to which attention was called in the previous stages. No statement as to the ultimate fate of this portion of the floor of the primitive nasal cavity can be made at this time. The cartilage contained in it is probably identical with what has been called the posterior transverse septum (Mead). Reference to figure 12 establishes, approximately, the position of the canal of Stenson in the earlier stages—a point between the bone of the maxillary and premaxillary palate.

SUMMARY

In its essential features the development of the nose in the spermophile does not differ from that of other mammals in which it has been described. The primitive nasal cavities develop as a pair of ingrowths from the region of the olfactory placodes.

A short distance in front of its blind posterior extremity each cavity grows toward the primitive buccal cavity (8-mm. embryo). Later it has extended toward the buccal cavity at this point until it remains separated from the buccal cavity by a thin membrane which consists only of the buccal and nasal epithelium. These abutting epithelial layers form what is known as the bucconasal membrane. The membrane ruptures at about the 10-mm. stage and the primitive choanae are thus established. At their first appearance the primitive choanae are located nearer to the anterior nares than to the buccal portion of the hypophysis. This statement is made

as locating the choanae somewhat more definitely than that given for man by Schaeffer as "well in advance of the buccal pituitary outgrowth in the roof of the mouth."

At about the time of the formation of the primitive choanae, there is evidenced a piling up of the epithelium lining the anterior nares, resulting in a diminution and finally in complete occlusion of the anterior nares by an epithelial plug. This plug persists until the closure of the canal of Stenson, when it shows signs of disintegration, and is finally destroyed and the lumen of each anterior naris reestablished.

Following the rupture of the bucconasal membranes, the primitive choanae thus formed elongate anteroposteriorly, due to a gradual recession of their posterior margins, i.e., the floor of the primitive nasal cavities posterior to the primitive choanae. The anterior margins of the primitive choanae maintain their original relative position, any apparent posterior migration of them being rather a growth outward of the face structures. The recession of the floor of the primitive nasal cavity (posterior margin of primitive choana) continues until retarded by the development within it of a lamina of cartilage derived from the cartilaginous nasal capsule. This floor remains, in the latest stage studied for this report, as a transverse septum separating the posterior portion of the primitive nasal-cavity derivative of the definitive nasal cavity from the buccal derivative of that cavity. The ultimate fate of this septum has not been established.

Coincident with the above changes, the palate processes of the maxillary process grow medially and inferiorly in the angle between the tongue and the floor of the primitive mouth. As a result of the general depression of the floor of the mouth and the increase in height of the maxillary processes, the tongue is freed from its position between the palate processes. It then becomes flattened vertically and spreads out in the floor of the mouth to occupy that portion of the mouth cavity formerly occupied by the palate processes. The latter grow medially above the tongue and fuse in the midline. This rotation and fusion of the palate processes incorporate a

small portion of the primitive buccal cavity into the definitive nasal cavity.

The fusion between the palate processes begins a short distance posterior to their anterior limits and progresses posteriorly. There remains between their anterior limit and the medial nasal process (anterior margin of the primitive choanae) a communication between the definitive buccal and nasal cavities, the canal of Stenson. This canal is later closed by the fusion of the medial nasal process and the palate processes. When the bone of the palate develops, the bony foramina of Stenson and Scarpa are formed at this point between the maxillary and premaxillary portions of the hard palate. The primitive choanae do not migrate posteriorly to become the permanent choanae; their posterior margins may migrate posteriorly to become the upper margins of the permanent choanae. Further work is necessary to decide this point, which depends on the fate of the remnant of the floor of the primitive nasal cavity forming the posterior boundary of the primitive choanae. The determination of the fate of this portion of the floor of the primitive nasal cavity involves a study of the cartilaginous nasal cavity.

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THE ENGULFMENT OF LIVING BLOOD CELLS BY OTHERS OF THE SAME TYPE

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ONE PLATE (SIX FIGURES)

In the course of our investigations on the behavior of the white blood cells in hanging-drop cultures of whole blood from the frog, we have occasionally noted engulfed living cells. The occurrence of ingested dead white cells within the large mononuclears was common, but the presence of living mononuclears within others of the same type was so rare that it attracted our attention at once. In the few series where it did occur there were many instances in almost every culture. As each series was made from a different animal, but otherwise in approximately the same manner, the indications are that this peculiarity was dependent upon some preexisting condition in the animal itself before the blood was drawn from the heart. As is well known, frogs are subject to various infections and parasites, and it may be that something of this nature predisposed the large mononuclear cells to exhibit this cannibalistic tendency in the hanging drops.

It is a rare phenomenon, and the only other case where we have observed it among thousands of cultures and spreads of living cells has been in pulmonary tuberculosis of the rabbit; here we have occasionally seen in living spreads loose epithelioid cells (hypertrophied monocytes) containing one and even two living epithelioid cells. These rare examples alone afford undoubted evidence of the existence of this process. The spreads from the tuberculous lungs where such

cells were found had been previously treated with neutral-red Locke solution and the cells observed had become detached from the small piece of tissue and were floating free in the surrounding fluid, where they were watched as they turned about in various ways. One could thus be absolutely sure that the engulfed cell was actually within the larger cell. The proof that the engulfed cells were living was the fact that they contained many granules that stained intensely with neutral red, and a nucleus that remained unstained. Ingested dead cells, on the contrary, stain diffusely, the nucleus usually more deeply than the cytoplasm. Observations on spreads from tuberculous lungs show that this process can take place within the body.

It is impossible to determine in fixed and stained tissue whether engulfed cells are or are not alive at the time of fixation. The only certain method for determining this question is by observation of the living cells.

Living white blood cells in hanging-drop blood and lymph-node cultures frequently come into intimate contact with one another without any indication of the engulfment of one by the other, and we have frequently observed small lymphocytes migrating about on the surface of a large epithelioid or giant cell without being engulfed. Such cells may, however, ingest many dead lymphocytes.

The factors which produce the extraordinary change in the monocytes, enabling them to engulf living cells, are totally unknown, but they undoubtedly are pathological and are probably responsible for the fatal course of certain types of anaemia.

OBSERVATIONS ON CULTURES OF FROG'S BLOOD

In one series (15-10-24), where the phenomenon of engulfment of living cells was especially noted, there were on the ninth day many large monocytes or epithelioid-like cells, often in clusters, that were quite heavily laden with neutral-red granules. The cluster shown in figure 1 was photographed after the cells had been stained with neutral red and while

they were still alive. Most of the blood was washed off with a neutral-red Locke solution, and only those cells that were stuck to the cover-glass remained. The neutral-red granules (black in the photograph) occupy most of the cell body. The nuclei (*1* to *3*) are at or near the periphery of the cell and are clear unstained oval bodies. In spite of the fact that these cells were heavily loaded with granules, it was possible sometimes to observe in the center of the cell a radial arrangement of them about a central point, presumably the centrosome. Similar cells from younger cultures of other series, which have become more extensively flattened out on the cover-glass and which contain fewer granules, show a radial arrangement of mitochondria about the centrosome. The mononuclear cell shown in figure 2 is from a six-day culture that had been washed with Janus-green Locke solution and then fixed with iodine vapor.

The granules that ordinarily take up neutral red are not stained as deeply by the iodine as the mitochondria. The latter are clearly seen and have a radial arrangement about the centrosome.

In the upper part of figure 1 there is a large giant cell, with three or four nuclei, which has engulfed a medium-sized living cell of the same type. The latter is enclosed in a large clear vacuole, the contents of which did not take up the neutral red. As can be seen in the photograph, the engulfed cell is packed full of neutral-red-stained granules and its clear nucleus lies at the periphery.

We have not actually watched the engulfment of such living cells, but various stages in what appears to be the process were found in this same culture. In figure 3 is to be seen the beginning of the process. Here the larger cell (*a*) is flowing, as it were, around the smaller cell (*b*). The surfaces of the two cells are in intimate contact without any fluid between them. Figure 4, from the same culture, shows an engulfing cell extending more than half-way around a large binucleate cell. Both cells are richly supplied with neutral-red-staining granules. Figure 5 shows a freshly engulfed living cell.

Our reason for considering this as having been recently engulfed is the fact that there is very little indication of fluid about it. Since the two previous figures indicate that the two cells are directly in contact during the engulfing process, we have naturally concluded that this same relation would prevail for some time after complete engulfment. How long a time such a relationship persists, we cannot tell, but it is apparent that the engulfed cell ultimately becomes surrounded by fluid, as in figures 1 and 6. This fluid is different from that in the ordinary vacuoles in that it does not take up the neutral red. The ordinary vacuoles are either digestive or degenerative. In the latter case they arise from autodigestion and accumulation of waste products or some associated process. The fact that the fluid surrounding the engulfed living cells does not take up neutral red indicates that it is not digestive in nature or does not contain substances that have a special affinity for neutral red. It is evident that neutral red passes through this fluid, for it accumulates in the granules of the engulfed cells.

Since these engulfing cells are against the cover-glass, it might be supposed that the engulfed cells are not really within, but merely between them and the cover-glass. Careful examination, however, revealed the fact that the engulfed cells and the fluid about them were completely surrounded by a thin layer of cytoplasm. The non-stainability of the fluid of the vacuole by neutral red and the fact that the engulfed cells are alive suggest that the process of engulfing is here somewhat different from that of ordinary phagocytosis, in that the lining of the vacuole corresponds perhaps to, and was originally a part of, the external surface of the engulfing cell. In other words, these engulfed cells are not taken into the cytoplasm. In ordinary phagocytosis that part of the external surface which surrounds the ingested particle is supposed to disappear or become transformed into ordinary interior cytoplasm by liquefaction at the place where the ingested particle enters. It would be very difficult, however, to prove definitely the existence or absence of a thin film of the external layer of the cell about ordinary ingested particles.

We have occasionally noted an engulfed living cell containing another engulfed living cell. The method by which this is accomplished is probably similar to that already noted for the engulfing of one cell by another. It is possible that the condition shown in figure 6 represents the manner in which this takes place. The large mononuclear cell (*a*) with a large vacuole contains a living engulfed cell (*b*). The cell (*a*) is in turn partially surrounded by two cells (*c* and *d*) which are spreading around it. Had one of the latter completely spread around the cell, the condition noted above would have been obtained. Occasionally a living cell may contain two engulfed cells; sometimes they appear to be in a common vacuole, sometimes in separate vacuoles.

Our observations are too fragmentary to enable us to determine at present the fate of the engulfed living cells. The occurrence of this phenomenon has only accidentally been observed, and no attempt has been made to control it and produce it at will. As already noted, the phenomenon is probably dependent upon the condition of the blood before it was withdrawn from the heart. One of the surprising things about the process is the apparent lack of any tendency of fusion between the engulfed and engulfing cells, in spite of the fact that they are closely attached during this process and have every opportunity for fusion.

EXPLANATION OF PLATE

All the figures except figure 2 are from a nine-day living culture of frog's blood that was washed in neutral-red Locke solution. The living cells are full of deeply stained red granules which photograph black. The clear unstained nuclei are at or near the periphery. The magnification for all figures is 700 diameters.

1 Living large mononuclear cells. About the cluster are distorted red blood cells. The giant cell *a* contains an engulfed living cell of the same type.

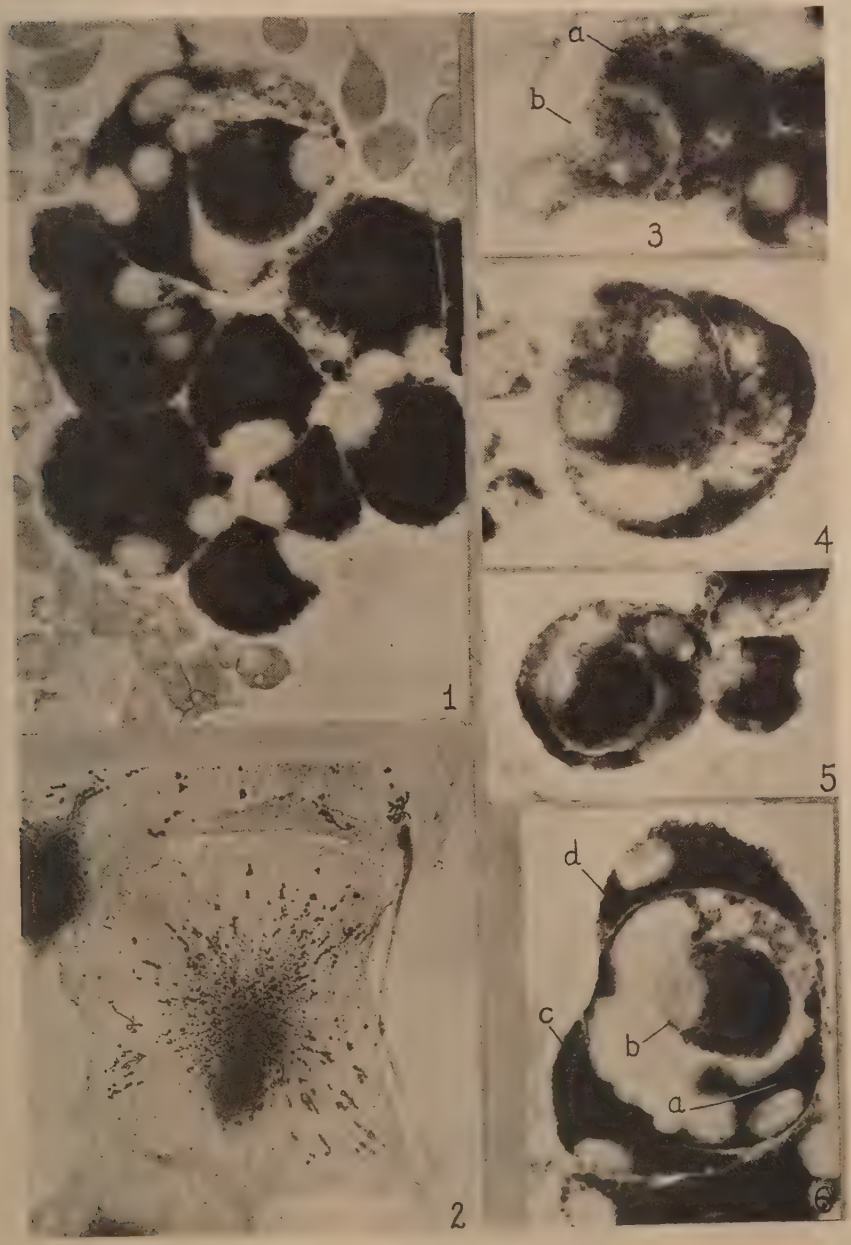
2 Large mononuclear cell spread out on the cover-glass. The mitochondria are more or less radially arranged about the centrosome. Six-day culture, Janus green, iodine vapor.

3 Showing the beginning of the process of engulfment of one living cell by another of the same type.

4 More advanced stage of engulfment of one living cell by another, the latter with two nuclei.

5 Freshly engulfed living cell.

6 Mononuclear cell (*a*) with a large non-stainable vacuole containing a living cell (*b*). The cells *c* and *d* appear to be surrounding the cell *a*.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VARIETIES AND THE SIGNIFICANCE OF GIANT-CELLS

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This contribution is the result of an effort to coordinate the widely scattered observations, and the diverse claims and suggestions, regarding the various types of giant-cells of mammalian and higher vertebrate tissues. The original stimulus for my investigations ('18, '20) of this subject was furnished by the persistent confusion, in certain text-books and portions of the literature, concerning the two distinct series of superficially similar giant-cells found closely associated in the early stages of enchondral bone development: the hemogenic ('megalokaryocytes') and the osseophagic ('osteoclasts'). More recently, further confusion has been introduced into this field by attempts to identify phagocytic giant-cells, particularly the osteolytic variety, with degeneration syncytia.

The main results of this study are summarized in the accompanying classification:

Giant-cells

- A. Hemogenic ('megalokaryocyte')
  - *Origin:* hemoblasts, endothelium
  - *Location:* yolk sac, liver, red bone-marrow, spleen, thymus, lymph-nodes
  - *Function:* blood-platelet ancestor; multiple erythroblasts
  - 1. Mononucleated (megakaryocytes)
  - 2. Polymorphonucleated (polymorphokaryocytes)
  - 3. Polynucleated (polykaryocytes)
- B. Phagocytic
  - *Origin:* mesenchyme, reticular cells, osteoblasts, osteocytes, lymphocytes, hemoblasts, endothelial cells
  - 1. Osteolytic—osteoclasts
  - 2. Chondrolytic—chondroclasts
  - 3. Odontolytic—odontoclasts
  - 4. Foreign body

  (multinucleated)
- C. Degeneration syncytia

The data tabulated are derived from many sources, for the most part fully indicated in the appended bibliography. The chief claim to originality pertains to the selection from among the mass of conflicting observations and conclusions those that now seem most closely to represent the actual genetic relationships. This study has, moreover, brought to sharper focus two additional facts of wide general significance: that of the high degree of functional adaptability on the part of the mesenchymal derivatives and that of the enhancement of phagocytic capacity following the event of cell fusions. These points will be discussed in detail below.

A. HEMOGENIC GIANT-CELLS

These are the so-called 'megalokaryocytes' of red bone-marrow. They occur in three chief modifications, classified on the basis of nuclear shape: the mononucleated, the polymorphonucleated, and the polynucleated. For purposes of brevity in nomenclature, these may be designated megakaryocytes, polymorphokaryocytes, and polykaryocytes, respectively. This order corresponds with the degree of progressive differentiation. The large, spheroidal, sharply contoured nucleus of the mononucleated hemogenic giant-cell becomes extensively lobulated in the typical polymorphokaryocyte with the so-called 'basket nucleus.' Subsequently, the delicate connecting threads of chromatin between the separating lobules may rupture and the giant-cell becomes multinucleated. In this condition in routine preparations, this cell is with difficulty distinguishable from the similarly multinucleated osteoclast.

This close similarity has led certain investigators mistakenly to identify these two series of cells, and to ascribe to the whole group the property of phagocytosis (Dickson, '08). Such interpretation of the multinucleated hemogenic giant-cells on the part of Schaefer ('10) accords with his description of blood platelets as nucleated elements, unrelated to cytoplasmic fragmentations of polymorphokaryocytes as described by Wright ('10). Certain investigators (Van

Bambeke and Van der Stricht, '92; Levy, '20) have claimed that the order of development of the giant-cells of the hemogenic series is the reverse of the one above given. They interpret the polykaryocytes as the younger, explaining the multinucleated condition as the result of multipolar mitoses. Subsequent partial and ultimately complete fusion of the several discrete nuclei would lead through the stage characterized by a polymorphous nucleus to the typical mononucleated condition. For the latter cell the term megakaryocyte would seem appropriate and useful. Etymologically, 'megalokaryocyte' has the same meaning, but since this word has been preempted as a generic term designating the entire hemogenic group, its use should be discontinued.

That the order of differentiation is from megakaryocyte to polykaryocyte, through polymorphokaryocytes, is indicated by the progressive increase in size of the cells correlative with lobulation of nucleus and by a corresponding loss of nuclear and cytoplasmic characteristics of cell youth, namely, vesicular nucleolate nucleus and basophilic cytoplasm, respectively. Small 'giant-cells,' with polymorphous pycnotic nucleus and meager envelope of oxyphilic cytoplasm, are such as have suffered a reduction of their cytoplasm through platelet formation. Such cells can be followed through transition stages to a condition in which they exist as naked disintegrating nuclei. Furthermore, the size variations of the nuclei of the polykaryocytes argue against a mitotic derivation.

Hemogenic giant-cells are found wherever hemopoiesis occurs; thus, first in the yolk sac, subsequently in the liver, spleen, thymus and lymph nodes, and throughout fetal and postnatal life in the red bone-marrow. After early fetal life these cells are practically absent from the liver and occur normally only very sparingly in the lymphoid organs. These giant-cells originate chiefly from hemoblasts, in small part from endothelial cells of the terminal capilliform sinusoids. Howell ('90) derives these cells by growth of 'small lymphoid cells.' Since hemoblasts themselves have a double origin

from mesenchyme and endothelium, an endothelial origin of hemogenic giant-cells is essentially the same as a hemoblast origin.

These are the cells that produce the blood platelets, as first demonstrated by Wright ('10). Moreover, in the yolk sac they may function in part as multiple erythroblasts ('16). They are, in fact, giant hemoblasts, and in the multinucleated condition they are comparable to blood islands. As polykaryocytes they differentiate erythrocytes intracellularly by a process involving the elaboration of hemoglobin about each nucleus. Each perinuclear halo of hemoglobin-containing cytoplasm becomes circumscribed by a membrane, and the erythrocyte thus differentiated comes to lie free in a vacuole. Subsequently, the giant-cell breaks up and liberates the several erythrocytes. This process of erythrocyte differentiation generally does not involve all of the nuclei at the same time. Occasionally, also, one nucleus with the peripheral shell of giant-cell cytoplasm does not undergo erythrocyte differentiation, but persists essentially unmodified as a structure comparable to an enveloping endothelial cell. At this stage the hemogenic giant-cell resembles a group of erythroblasts within an endothelium-lined lumen. Such a process of intracellular giant-cell production of erythrocytes has been described also by Foa ('99) in bone-marrow. Pugliese ('97) and Denys ('86) have described both erythrocytes and leucocytes originating in these giant-cells. From the viewpoint, then, of its significance with respect to platelet ('19) and potential erythrocyte production, this giant-cell is appropriately designated 'hemogenic.'

B. PHAGOCYTIC GIANT-CELLS

The phagocytic series of giant-cells is uniformly multinucleated. In the last analysis the cells of this series include as many types as the number of different substances which they phagocytose. Among these types are most prominently the osteolytic, chondrolytic, and odontolytic giant-cells. They may be more briefly designated, respectively, osteoclasts

(Koelliker, '72; 'myeloplaxes' of Robin), chondroclasts, and odontoclasts. Here may be mentioned also cementoclasts and the ameloclasts. Another group of this division are the so-called 'foreign-body' giant-cells of pathologic and experimental tissues. In fact, the whole series of phagocytic giant-cells are essentially foreign-body giant-cells. The nomenclature sufficiently indicates the function of the several subdivisions of the phagocytic giant-cells.

For immediate purposes we may confine our attention to the osteoclasts. These are the giant-cells which are confused with polykaryocytes of the hemogenic series. They are both similarly multinucleated, and, in routine preparations, have a practically identical staining reaction. The next point, then, concerns the differentiation between hemogenic polykaryocytes and osteoclasts, as they occur closely associated during the early stages of enchondral bone formation. The differential criteria can best be treated under four heads: location, staining reaction, origin, and function.

The osteoclasts are almost invariably located closely adjacent to spicules of primary bone; frequently occupying shallow pits, the so-called 'Howship's lacunae,' presumably the result of a resorptive action on the part of these giant-cells. The hemogenic giant-cells, on the contrary, lie within the primary marrow areolae, almost invariably at an appreciable distance from the osseous trabeculae.

From the standpoint of certain staining reactions, also, the hemogenic contrast sharply with the osteolytic giant-cells. Probably the sharpest color contrast is obtained with the Wright's technic, applied according to the method first employed in the study of the hemogenic giant-cells as sources of platelet supply. As Wright's illustrations beautifully show, these cells are characterized by a granular endoplasmic area of deeply red or lilac color, enveloped by a slightly basophilic exoplasm. Pseudopods contain these same two areas, and segment into platelets each with an endoplasm and exoplasm of these respective materials. The osteoclasts under this technic reveal no such differentiation into exoplasm and

endoplasm. Their cytoplasm during the younger phases is faintly basophilic; subsequently it becomes decidedly oxyphilic. This more or less rapid assumption of oxyphily is presumably the result of the cooperation of two closely related factors: resorption of semifluid degenerate osseous material, with a consequent dilution of the cytoplasm, and a chemical change in the protoplasm incident to intense functional activity and age. Moreover, the nuclei of these cells are generally clearer in appearance, with a more delicate reticulum, than are those of the hemogenic giant-cells. Furthermore, with certain technics, particularly fixation with strong Flemming's fluid followed by the iron-hematoxylin staining procedure, the hemogenic giant-cells disclose large pluricorpuscular centrosomes, whereas no centrioles are discernible in the osteoclast cytoplasm.

In origin the two series of cells differ widely. The hemogenic polykaryocytes are giant multinucleated hemoblasts. They become multinucleated in consequence of repeated amitotic division of the originally single nucleus. The whole group of phagocytic giant-cells, on the contrary, become multinucleated as the result of fusions of originally discrete cells. These cells may be of various sorts: mesenchymal, reticular, osteoblasts, lymphocytes, hemoblasts, endothelial cells. The fusion products may include only one type of cell or several types. For example, in the earliest stages of osteogenesis generally, only reticular cells fuse to form osteoclasts. During later stages osteoblasts contribute to the definitive osteolytic syncytia, or osteoclasts may arise exclusively from fused osteoblasts. When the primary bone begins to be resorbed and osteocytes are set free, these also may fuse with preformed osteoclasts. Possibly this process, however, is more correctly interpreted as a mode of phagocytosis. The nuclei of the ingested osteocytes may become indistinguishable from the proper nuclei of the osteoclasts. Under certain conditions, endothelial cells and lymphocytes also may contribute to the osteolytic giant-cells.

Under specific experimental conditions, hemoblasts also may fuse to produce typical osteoclasts ('25 c). And Mallory ('14) is of the opinion that all foreign-body giant-cells arise only from fused endothelial cells.

The chondroclasts, through whose activity the hyaline cartilage is resorbed during the early stages of substitution bone formation, are exclusively of reticular origin ('25 c). The odontoclasts (cementoclasts) are fused masses of either fibroblasts or endothelial cells, or partial derivatives from both sources. The ameloclasts, the giant-cells that remold the young enamel, especially of the molar teeth, are apparently unique in that they arise by fusion of ectodermal cells of the enamel pulp ('23), though endothelium cannot be entirely excluded from possible participation. In the initial stages of the earlier fusions of reticular cells an occasional nucleus may divide by mitosis, but subsequently either mitotic or amitotic nuclear divisions in these giant-cells are of the very rarest occurrence, if indeed they happen at all.

The specific functions of the two series of giant-cells have been briefly indicated above: the hemogenic giant-cells of the hemopoietic tissues produce platelets, and, at least in the yolk-sac wall, they function in small degree also as multiple erythrocyte ancestors ('18); the phagocytic giant-cells have exclusively a resorptive function, ingesting and digesting cartilage, bone, cementum, effete erythrocytes, and foreign bodies generally, including microorganisms.

The terms phagocytosis, resorption, ingestion, and digestion are here used practically synonymously. In the complete function of these cells, these specific phases merge one into the other. For example, in certain instances where redundant bone may be caused to liberate semisolid globules in the osteolytic process, these globules may be phagocytosed (ingested) as such, and only subsequently become resorbed (digested) intracellularly ('21); in the more general case where the bone is first rendered fluid by the lytic enzyme liberated by the giant-cell, or the general reticular stroma, the 'digested' bone is resorbed in the shape of fluid by the

phagocytic giant-cell. The structural manifestation of this resorptive process inheres in the 'striated border' of the giant-cell located only on the side next the layer of bone or cartilage.

C. DEGENERATION SYNCYTIA

A third class of giant-cells must be recognized, namely, the degeneration symplasms. These are fused masses of degenerating cells. While the hemogenic and phagocytic giant-cells, with a few possible exceptions among the latter (e.g., ameloclasts), are of mesodermal derivation, the degeneration syncytia arise from cells of any of the three germ layers. A common example of such plasmodial masses of degenerating cells are those composed of fused seminal cells in the tubules of undescended testes. Similar masses may occur by passive fusion of cells at any level of the male genital-duct system under inflammatory conditions. The chief factors concerned in the production of this type of giant-cell would seem to be the enhanced adhesive property of the cytoplasm of degenerating cells and their location within exiguous confines.

The importance of specially recognizing this type of giant-cell results from the confusion in certain quarters between these and true phagocytic giant-cells, especially the osteoclasts, and the denial to both alike of any direct resorptive function, and the ascription to both alike of only a degenerative significance. Thus Arey ('20) interprets osteoclasts as fused depleted osteoblasts. Kingery ('24) even more definitely describes them as fusion products of degenerating cartilage and bone cells set free by the liquefaction of cartilage and bone, but nevertheless he ascribes to them a possible osteolytic function inherent in a hypothetical enzyme liberated in the process of disintegration. By implication, Macklin ('20) likewise regards osteoclasts as degeneration products. In the transformation of experimental callus in the rat he attributes the bone and cartilage resorption solely to macrophages derived from reticulum cells. In contrast to the

behavior of the osteoclasts, the macrophages stained brilliantly with trypan blue. The number of osteoclasts is said not to bear any relation to the apparent amount of bone destruction going on in the change of provisional into permanent callus.

DISCUSSION

The reason for this identification of osteoclasts with degeneration syncytia results from the consideration of osteoclasts only during their later stages, and a neglect of the earlier stages. For a brief period after formation, the length apparently varying with the rapidity of function, these cells have the general nuclear and cytoplasmic characters indicative of healthy cells. With appropriate technic an abundance of mitochondria can be demonstrated in these cells during early stages. Due to the relative intensity of their phagocytic activity, they rapidly pass into degenerative conditions, and ultimately disintegrate. The observation that both bone and cartilage, and foreign materials generally, can be resorbed in the absence of giant-cells cannot be legitimately urged as contradicting the conclusion that phagocytic giant-cells, as such, exercise a resorptive function. That discrete mesenchyme cells, and their early derivatives, perform various resorptive and phagocytic functions is abundantly attested by numerous direct observations ('25 c). However, that giant-cells, formed by fusions of such mesenchymal elements, likewise have similar resorptive capacity, and apparently in enhanced degree, seems equally clear. This matter will be more fully treated after a further consideration of the point concerning the variable mode of origin of osteoclasts.

At first thought it seems especially paradoxical that osteoblasts, whose specific function is one of bone secretion, should, after fusion into syncytia, assume the reverse function, namely, that of bone lysis. And it seems at first even more startling to attribute to fused masses of hemoblasts a bone-eroding function, when these cells, as discrete elements, clearly function as blood-cell ancestors. The facts, however,

are unmistakable. In the experimental red marrow of splenectomized frogs, contrary to the posthibernation red marrow of unoperated frogs, enormous numbers of osteoclasts appear in the shaft of the femur by fusion of hemoblasts. As already explained ('25 c), this unique phenomenon most likely finds its correct interpretation in the fact that, the reticular stroma having already been largely preempted in the process of hemoblast origin, osteolytic giant-cells must be formed by fusions of the only adequately available type of cell, namely, the hemoblast.

The key to the riddle of this multiple origin of phagocytic giant-cells is the pluripotential developmental capacity of mesenchymal cells and their early differentiation products, among which is included prominently a phagocytic and resorptive function. Mesenchyme may become actively phagocytic (Danchakoff, '21 a and b; Danchakoff and Seidlein, '21 and '22; Clark and Clark, '18). Reticular cells, as differentiated mesenchymal elements, have an especially high degree of phagocytic capacity (Wislocki, '21; Maximow, '24). Similarly, endothelial cells may express their potential phagocytic function. Under many conditions, lymphocytes become phagocytic (Downey, '12; Maximow, '24; Jordan, '24 and '25 b); similarly, osteoblasts (Nagao, '20; Wislocki, '21). Moreover, Danchakoff ('18) believes that hemoblasts have a phagocytic capacity for (abnormal?) erythroblasts.

Fusions of these several varieties of cells, already endowed with a certain degree of resorptive and phagocytic capacity, apparently effect a gain in efficiency and speed of phagocytosis. A parallel to this phenomenon in mammalian and higher vertebrate tissues is disclosed by the work of Kepner ('25) and his students among certain protozoa. Here, originally separate cells (e.g., Actinophrys) may cooperate in ingesting a larger food mass (e.g., a desmid), or in phagocytosing a hyperoptimum foreign particle, this cooperation occasionally involving an intimate transient fusion. However, in these instances among protozoa, the object of fusion would seem to be simply fixation and more complete envelopment

of the prospective food particle. It seems clear that foreign-body giant-cells likewise fuse about objects to be ingested, but fusion here apparently has a much deeper significance than mere envelopment, for it is resorted to much more extensively in instances when the giant-cell can come into relation only with one side of the foreign material, as in the case of the relation of the osteoclast to the plate of redundant bone. Frequently, the condition presented is one intermediate between envelopment and lateral invasion, as when an osteoclast surrounds the end of a trabecula of bone. Moreover, it must also be recognized that the formation of degeneration syncytia frequently involves adjacent effete blood cells and other débris. Misinterpretation of this condition is in part responsible for the confusion between phagocytic giant-cells and degeneration plasmodia.

The conclusion that fusion of mesenchymal cells effects a gain in phagocytic speed and efficiency is suggested by three striking recent observations. Danchakoff ('21) made grafts of the Ehrlich mouse sarcoma, mixed with fragments of adult-chicken spleen, upon the allantois of the seven-day-chick embryo. Both sarcoma and splenic tissue proliferated within the mixed graft. Sections of the graft of four and five days' growth showed numerous sarcoma cells, each closely enveloped by a capsule of fused mesenchymal cells. Such encapsulated sarcoma cells showed decided regressive changes indicative of rapid digestion.

Maximow ('24) inoculated with human tubercle bacilli tissue cultures of rabbits' lymph nodes in blood-plasma clots. Under these conditions, typical giant-cells laden with bacilli formed in the cultures. Ingestion of bacilli was accomplished also by reticular cells, lymphocytes, and monocytes ('polyblasts'). The giant-cells of these cultures formed only from fusions of reticular cells and lymphocytes. These giant-cells are comparable to the Langhans' cells of tuberculous nodules, formed, according to Mallory ('14), by fusions of endothelial cells; according to Warren ('17), by fusions of reticular cells. Maximow makes the significant statement that "In the giant cells, the digestive power of the protoplasm is probably far

higher, and therefore the number of the intracellular bacilli usually does not increase excessively'' (p. 32).

Experimental abdominal hernia of the ovary in the frog is rapidly repaired by ingrowth and overgrowth from the surrounding skin. As briefly described previously ('25 b), the larger eggs become completely encapsulated with a several-layered envelope of rounded mesenchymal cells. The oval yolk grains become broken up into spheroidal granules of various sizes and pass into the encapsulating 'giant-cell.' Under normal conditions, the eggs which remain after the breeding season are also resorbed, but without the formation of distinct enveloping syncytia. The speed of egg resorption under normal and experimental conditions is closely correlated with the mode of mesenchymal activity, that is, whether by unmodified mesenchyme or by giant-cells.

At this point it seems necessary to return to the question of the method by which the phagocytic giant-cell attains its multinucleated condition. In the vast majority of cases multinuclearity in these cells is certainly the result of fusions of discrete cells. Except for the very earliest stages, when nuclear mitosis and amitosis may play a small part, the number of nuclei increases only by additional fusions. Regarding the giant-cells ('Langhans' cells') found in plasma cultures of tuberculous and normal human lymph nodes, Lewis and Webster ('21), though uncertain as to the actual origin of these cells, incline to an interpretation of their multinucleated condition on the basis of amitotic nuclear divisions in enlarging wandering cells. More recently, Lewis and Lewis ('25) have touched the same point in their description of giant-cells of the Langhans' type in a twelve-day culture of lizard's blood. In this and similar cultures of various bloods, leucocytes could be seen to transform into macrophages, epithelioid cells, and giant-cells. They find no evidence whatever that these giant-cells arise by fusions of mononuclear epithelioid cells. They state that ''They probably arise by division of the nuclei without division of the cytoplasm.'' However, the very close uniformity of nuclear size and morphology, viewed in the light of abundant unquestionable

evidence that similar giant-cells arise from cell fusions—coupled with the admitted uncertainty regarding the origin of the multiple nuclear conditions—makes permissible considerable skepticism regarding the intervention of nuclear divisions.

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STUDIES OF HYPERTHYROIDISM

I. EXPERIMENTAL HYPERTHYROIDISM AND THE GERM GLANDS IN AMPHIBIANS, WITH SPECIAL REFERENCE TO THE PRIMITIVE INTERSTITIAL TISSUE

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ONE PLATE (FIVE FIGURES)

INTRODUCTION

The possibility that the active principle of the thyroid gland may exert an influence upon the condition and state of development of the sex glands has long been recognized by various investigators. The results of thyroidectomy upon young animals and the effects of various thyroid diseases and anomalies have suggested some relation between the thyroid autacoid and the sex glands. For this reason the negative results of Allen and Swingle are somewhat unexpected and surprising.

Allen ('18) has studied in frog larvae the effect on the gonad of thyroidectomy—an operation which presumably leads to a condition of hypothyroidism. Complete thyroidectomy, he finds, though it markedly retards differentiation of the body, does not prevent complete differentiation of the gonad. He concludes that "the removal of the thyroid gland has no effect of any kind upon the gonads and germ cells," and that "this is in significant contrast to the effect upon all the other portions of the body." Hoskins and Hoskins ('19), working independently of Allen and publishing about a year later, do not agree with him that thyroidectomy demonstrates quite so sharp a difference be-

tween the gonads and the remainder of the body. Although they find that mature spermatozoa (but not mature ova) may be produced in a thyroidectomized tadpole in which bodily differentiation is greatly retarded, they are not willing to go so far as Allen. They compromise somewhat with the statement that the growth and differentiation of the germ glands are to a limited extent independent of the growth and differentiation of the body as a whole.

Swingle ('18) has studied in frog larvae the effect on the gonad of thyroid feeding, which leads presumably to a condition of hyperthyroidism. He finds that the gonads are not perceptibly affected by this treatment, and concludes that "the thyroid gland has no intimate physiological relation to the gonads and germ cells in amphibia."

In brief, the experiments on frog larvae indicate that mature germ cells (spermatozoa) may be produced in the absence of the thyroid gland, and, conversely, that the precocious general metamorphosis induced by thyroid administration does not include the precocious maturing of the germ gland. The idea has, therefore, become more or less prevalent that the germ glands are relatively distinct from the rest of the body in the response to the thyroid autacoid. None of the investigators in this field mentions any effect upon the mesenchymal interstitial cells of the germ glands.

From histological studies of the effects of experimental hyperthyroidism, the writer ('24) had already reached the general conclusion that the thyroid autacoid has a primary selective effect upon the undifferentiated cells of the body causing increased rate of proliferation. This increased rate of proliferation of undifferentiated cells is one aspect of the general rise in metabolic rate known to occur in the condition of hyperthyroidism. It seemed improbable that the gonads of frog larvae, which contain undifferentiated cells, both germinal and interstitial, should show no response to the hyperthyroid condition. Accordingly, the subject was reinvestigated with the special view of searching not for acceleration of differentiation or maturation, but for acceleration of cell proliferation.

MATERIAL

In the first series of experiments, tadpoles of the green frog (Rana clamata) obtained near Charlottesville, Virginia, were used. Most of these were collected and used in December, a few in January and February. In length they varied from about 4 to 6.5 cm. Metamorphosis was accelerated by placing desiccated extract of sheep thyroid in the water with the animals. The results recorded here are based largely upon examination of the gonads of forty-five tadpoles of this series. Of these, twelve were normal untreated controls, eight females and four males. Of the remaining thirty-three thyroid-treated individuals, nineteen were females killed at intervals of two to fifteen days after the first thyroid administration, and fourteen were males[1] killed at intervals of two to fifteen days after the first thyroid administration.

In the second series of experiments, tadpoles of the wood-frog (Rana sylvatica) collected at Woods Hole, Massachusetts, in the latter part of June were used. These were close to the time for metamorphosis. In fact, a couple of animals (which were discarded) had already protruded the fore limbs at the time the collection was made. Both in the normal control group and in the thyroid-treated group, eruption of forelimbs began to take place within two days. In the thyroid-fed group of forty animals, all had protruded fore limbs within five days. In the normal control group of forty animals, there were still left four animals without fore limbs after ten days. General metamorphic change was noticeably more rapid in the thyroid-fed animals. Individuals from each group were killed or died at intervals ranging from two days to thirty-two days. Under laboratory conditions that were not ideal, the mortality was found to be greater among the normal control animals than among the thyroid-fed animals.

In the third series, salamander larvae of the species Amblystoma punctata were used; forty-three for thyroid treatment,

[1] In this number is included one hermaphrodite, the gonads, however, being predominantly of the male type.

seventeen for normal controls. These were collected and used at Woods Hole in the latter part of June. Individuals from each group were killed or died at intervals ranging from one to twenty-eight days.

OBSERVATIONS

The testis

The testis of the Rana clamata tadpole in the stage of development used consists of seminiferous tubules, and between these, mesenchymal interstitial cells (figs. 1 and 2). The lining of the seminiferous tubule is two or three cell layers thick with two distinct cell types. One type has a large vesicular, lightly staining nucleus which is usually lobulated; the other has a smaller deeply staining nucleus and little cytoplasm. The latter type is usually at the luminal portion of the tubule, the former at the basal portion of the tubule. Wandering lymphoid cells may be seen occasionally invading the tubule. Sloughing off of cells into the lumen occurs, detached cells being often visible in the tubule cavity. An occasional mitotic figure may be found, but nearly all of the cells, both germinal and interstitial, are in the resting stage.

The most striking effect of thyroid treatment upon the testis is an increase in the mitotic activity of the intertubular mesenchymal cells—the forerunners of the interstitial cells. Proliferation of these proceeds at a rate quite definitely higher than the normal rate. This is well seen several days after the first thyroid administration (figs. 2 and 3). In later stages of thyroid treatment the proliferative activity is not maintained at the high level, but decreases, very little taking place after thirty days. This later decrease may be associated with general inability on the part of the animal to keep pace with the increased metabolic demands.

A less conspicuous increase in the proliferative rate of the spermatogonial cells by mitosis also occurs (fig. 2). In the later stages of thyroid treatment this rate falls back toward the normal.

In the larvae of Rana sylvatica little difference could be noticed in the division rate of either mesenchymal interstitial cells or germinal cells. If there is any difference, it is in favor of the thyroid-treated animals. It should be noted that these animals were on the verge of metamorphosis at the time the experiment was started. In the normal controls, therefore, the concentration of thyroid secretion from the thyroid gland was great enough to be operative. The mesenchymal interstitial tissue was much less in amount than in the tadpoles of Rana clamata, and therefore not so favorable for comparative observations.

In the case of Amblystoma, the proliferation rate of the germ cells appeared to be somewhat greater in the thyroid-fed animals than in the normal controls. The gonads, however, were very small, so that no large numbers of cells were present for observation. On account of the meagerness of the interstitial tissue, no conclusion concerning proliferation rate of these cells was drawn.

The ovary

The bulk of the ovary of the normal untreated tadpole in the stage of development used consists of fairly large definitive oocytes, already containing a certain amount of reserve food material (fig. 4). Each oocyte is surrounded by a cellular follicular membrane. A relatively meager interstitial tissue is present. The germinal epithelium is at the periphery, and varies somewhat in different individuals in the number of cells composing it. An occasional mitosis may be seen in the germinal epithelium, but mitoses are relatively rare. In some ovaries (usually at one pole) there may also be seen cells in process of maturation.

The ovary of a thyroid-treated animal presents definite, though by no means striking, differences (fig. 5). The proliferative rate of the germinal epithelial cells is increased, as is also that of the interstitial cells. More mitotic figures may be seen. The definitive oocytes and the follicle cells,

both of which are highly differentiated cells, are not perceptibly affected.

In ovaries of Rana sylvatica little difference is noticeable, this species being by no means as favorable for observation as Rana clamata.

In Amblystoma larvae the scarcity of the interstitial tissue prevented a definite conclusion. Division rate of the germinal cells appeared to be slightly increased in the thyroid-treated animals as compared with the untreated normal ones.

INTERPRETATION AND DISCUSSION

These observations upon the germ glands of tadpoles under conditions of experimental hyperthyroidism lead to the following conclusions: 1) There is little, if any, increase in the rate at which differentiation or maturation progresses in the germ glands during one month of treatment. 2) There is a definite, though not conspicuous, increase in the rate of proliferation of undifferentiated germinal cells, i.e., spermatogonia and, to a less degree, oogonia. 3) There is a striking increase in the rate of proliferation of the mesenchymal intertubular cells—the progenitors of the interstitial cells.

The first conclusion is in agreement with that of Swingle. It is based upon experiments upon tadpoles (Rana clamata) that were several months from the time of metamorphosis and upon tadpoles (Rana sylvatica) that were on the verge of metamorphosis. In the former species no difference in the rate of gonad differentiation was discernible; in the latter species a slight difference was seen in favor of the thyroid-treated animals, though this may readily be ascribed to normal variability. Caution is necessary, however, in interpreting this negative result. It must be kept in mind that the observations on animals under thyroid treatment cover only about a month. During the latter half of this period many of the younger tadpoles become somewhat abnormal, as if not quite able to keep up the pace initiated by the treatment. In these, respiration (Speidel, '25) and blood formation (Jordan and Speidel, '23) are both dangerously interfered

with. Under such conditions, the animals might not be expected to show progressive changes in the germ glands—organs that are by no means essential to the existence during or after metamorphosis. The energies of the metamorphosing tadpole would naturally be directed more or less toward effecting those changes vital to its continued life under the new land conditions. It still seems possible to the writer that the thyroid treatment may actually bring nearer or advance the time for gonad differentiation, though this is masked by the grave metabolic disturbances which occur after the first couple of weeks of treatment.

In regard to the second and third conclusions given above, it may be said that hyperthyroidism is known to be associated with increase in general metabolic rate. Undifferentiated cells all over the body of the tadpole respond to this by increased rate of mitotic proliferation. It is, therefore, to be expected that the undifferentiated cells of the germ glands would exhibit a similar response. As has been recorded, some increase in the division rate of spermatogonia and, to a less degree, oogonia does take place; also a conspicuous increase in the mitotic rate of the interstitial cells. These findings bring the germ glands in line with the rest of the body in the general response to hyperthyroidism. The gonads cannot be regarded as entirely distinct, as held by Allen and Swingle. Other cells in the germ glands show no perceptible histological changes, although presumably their rate of metabolism is increased. Of this kind are the oocytes and the follicle cells of the ovary. These are highly differentiated types and are the kind, therefore, in which cell division would not be expected.

Judging from the histological data, there is a certain similarity in the response to thyroid of the germ-gland cells and of the somatic cells of some organ, as, for example, the eye. In each case the highly differentiated cells remain unaffected. The undifferentiated cells are stimulated first to increased proliferative rate and later at the proper time, as determined by the complete hereditary complex, to dif-

ferentiation or maturation. It has been shown (Speidel, '24) that in the eyeball the ora serrata region of the retina is greatly affected by thyroid treatment, the basal layer of undifferentiated cells proliferating rapidly, while in adjacent portions of the retina or iris where the cells are already differentiated, no effect is perceptible. Likewise, in the germ glands the undifferentiated cells, both interstitial and germinal, are stimulated to proliferation, whereas the differentiated ones remain unaffected. Thus, since the ovary is composed for the most part of cells already highly differentiated, i.e., oocytes and follicle cells, there should be, and is, less mitotic activity than in the less highly differentiated testis.

Of special interest in this study is the response of the interstitial cells to thyroid treatment. An undoubted increase in the division rate of these cells occurs. The function of interstitial tissue in amphibians is not surely known. Humphrey ('21) has studied the seasonal variations in interstitial tissue in the testis in various urodele amphibians. In general, he finds that increase in interstitial tissue is correlated with regressive phases in the spermatogenetic cycle. He finds little evidence of secretion products other than lipoid substances, and is inclined to doubt that the interstitial cells have anything to do with secondary sexual characteristics. On the other hand, in frogs it has been found that the thumb pad in the male, of use in copulation and an undoubted secondary sexual characteristic, is dependent for its development on the testis. Castration in the young frog prevents its appearance, whereas testicular transplantation in the lymph sac of a young castrated frog will be immediately followed by its development (cited from MacLeod, '20). This means that a chemical substance produced in the testis is responsible for this secondary sex character. Either the interstitial cells or the tubule cells must produce this substance. There is, likewise, a difference of opinion concerning the function of interstitial cells in mammals. The extensive indirect evidence supports the idea that the interstitial tissue is probably the physical basis responsible for

sexual passion (Whitehead, '08) and probably for certain secondary sexual characteristics, though here, too, the possibility of influence of the tubule cells has perhaps not been unequivocably ruled out.

The fact that a hyperthyroid condition stimulates multiplication of the interstitial cells in the larval frog leads to the inference that a hypothyroid condition may be followed by a subnormal proliferation of these cells. Neither Allen, nor Hoskins and Hoskins, in their observations on germ glands after thyroidectomy, refer to these cells, possibly because of their relative scarcity in the species of tadpole used. In developing mammals conditions homologous to those in frog larvae may obtain. That is, a hypothyroid condition in the foetus may retard cell proliferation of the interstitial cells to such an extent that imperfect development of the reproductive apparatus and of secondary sexual characteristics may result. In like manner, a hyperthyroid condition in the foetus may stimulate cell proliferation of the interstitial tissue to such an extent that precocious development of the reproductive apparatus and of secondary sexual characteristics may result. Especially does this aspect seem important in view of the known fact that interstitial-cell mitoses are of most infrequent occurrence in the adult and even in the early life of a mammal, as pointed out by Allen ('04) and Whitehead ('04) in the pig, Allen ('04) in the rabbit, Duesberg ('18) and Jordan ('11) in the opossum, Rasmussen ('17) in the woodchuck, and Kasai ('08) and others in man. In other words, if normal multiplication of interstitial cells does not occur in the early developmental stages, this tissue may remain permanently inadequate.

Certainly, the often-observed correlation between hyperthyroidism and precocious sexual conditions and, conversely, between hypothyroidism and immature sexual conditions, does lend general support to the view outlined above. An early stimulus to the interstitial cells to proliferate, such as is given by experimental hyperthyroidism in frog larvae, or an early retardation of cell proliferation of this tissue, such

as takes place presumably after hypothyroidism, would seem to furnish a plausible physical basis for the production of the later-observed results. It is, of course, dangerous to combine results or observations in one group of animals with those in another group. Nevertheless, these data suggest a part of the correlation mechanism between variation in the thyroid autacoid on the one hand and sexual variation on the other.

SUMMARY

Experimental hyperthyroidism in frog larvae brings about increased rate of mitotic proliferation of the undifferentiated cells of the germ gland. This increase in mitotic rate is most conspicuous in the case of the interstitial cells. The spermatogonia and oogonia are stimulated to a somewhat less degree. These effects may be well seen about a week after the first administration of thyroid extract. The mitotic rate of highly differentiated cells, such as the oocytes and the follicle cells surrounding them, remains unaffected. The germ glands, therefore, in spite of the apparent absence of any acceleration of differentiation or maturation, can by no means be considered as being sharply distinct from the rest of the body in the general reaction to hyperthyroidism.

The effect of hyperthyroidism upon the primitive interstitial cells suggests a part of the mechanism by which early maturity in secondary sexual characteristics is attained by hyperthyroid individuals and, conversely, the mechanism by which retarded or late maturity in secondary sexual characters results in hypothyroid individuals.

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PLATE 1

EXPLANATION OF FIGURES

1 Longitudinal section through the testis of a normal untreated tadpole (Rana clamata). Three seminiferous tubules may be seen with the primitive interstitial tissue between. No mitotic figures are visible.

2 Longitudinal section through the testis of a thyroid-treated tadpole (Rana clamata), killed six days after the first administration. In each of the three seminiferous tubules may be seen a germ cell (*G*) in mitosis. Three interstitial cells (*I*) in mitotic division may also be seen.

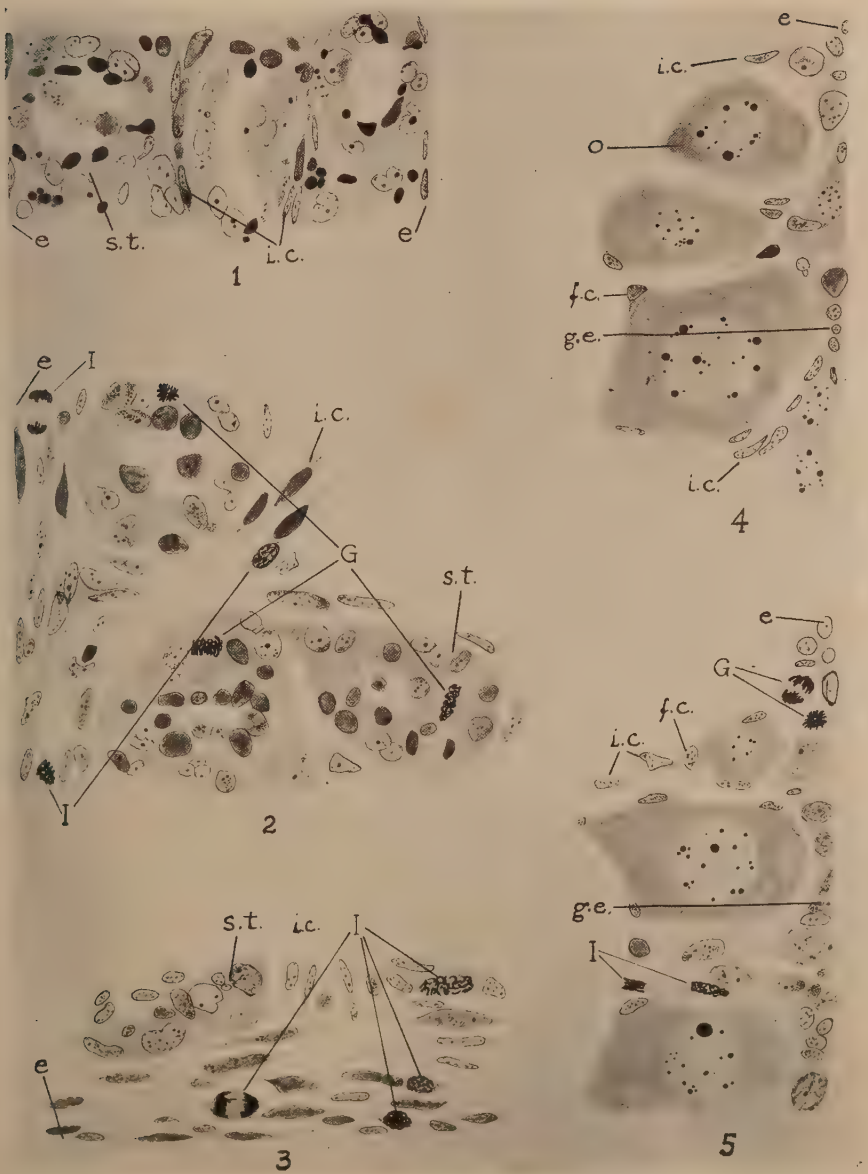
3 Longitudinal section through the testis of a thyroid-treated tadpole (Rana clamata), killed six days after the first thyroid administration. Four interstitial cells (*I*) may be seen in process of mitosis.

4 Longitudinal section through the ovary of a normal untreated tadpole (Rana clamata), showing germinal epithelium and oocytes of various sizes. A few interstitial cells in the resting stage are present; also follicle cells around the oocytes. No mitoses are visible.

5 Longitudinal section through the ovary of a thyroid-treated tadpole (Rana clamata), killed four days after the first thyroid administration. Two germ cells (*G*) and two interstitial cells (*I*) may be seen in mitotic proliferation.

ABBREVIATIONS

e., outer edge of the gonad
f.c., follicle cell
G., germ cell in mitosis
g.e., germinal epithelium
I., interstitial cell in mitosis
i.c., interstitial cell in the resting stage
o., oocyte
s.t., seminiferous tubule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A CASE OF ANOMALY OF THE LOWER END OF THE AORTA

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THREE FIGURES

The singularity of the case described below is due to the fact that the lumen of the aorta does not end at the point of bifurcation, but immediately beyond the medial ridge separating the common iliac arteries from each other, and extends into a groove from which arise the middle sacral artery and the fourth pair of lumbar arteries. At first sight, this formation resembles a small aneurysm. However, as will be shown later, the primary cause of this anomaly is irregularity of intra-uterine development of the lower end of the aorta.

The lower end of the aorta is a very complex formation from an embryological standpoint. According to Broman ('06, '08, '11), the definitive abdominal aorta is formed from the primitive aorta, the cranial end of the middle sacral artery, and initial portions of the common iliac arteries, which become fused over a certain length and form the caudal end of the definitive aorta. The anomaly here described seems to confirm Broman's theory of fusion of the common iliac arteries. To this we shall return later.

The middle sacral artery, which is also involved in this anomaly, at some stages of intra-uterine development forms the direct prolongation of the trunk of the aorta. Thus, in a human embryo 5 mm. and 9 mm. in length (Tandler, '04) or 8 mm. (Broman, '08) the middle sacral artery, prolongating the aorta directly backward, is in its cranial section of the

same caliber as the trunk of the aorta, and only farther back becomes narrower. In a 4-mm. embryo described by Bremer ('06) the sacral artery, judging by its direction, is evidently the prolongation of the trunk of the aorta; the caliber of its initial portion is not apparent from the figure. However, in earlier stages of development, namely, in an embryo 1.3 mm. long, described by Eternod ('98), each primordial aorta passes into its primordial umbilical artery; the caudal arteries are not existent at this stage. In this connection, Broman ('08, p. 540), basing his contention on comparative anatomy of vertebrates, thinks that phylogenetically, if not ontogenetically, the middle sacral artery of man was originally a caudal prolongation of the aorta. Young and Robinson ('98) are of a different opinion: they hold the common iliac arteries to be the caudal prolongation of the aorta and regard the middle sacral artery as a fused and caudally directed pair of dorsal segmental branches of the aorta.

DESCRIPTION

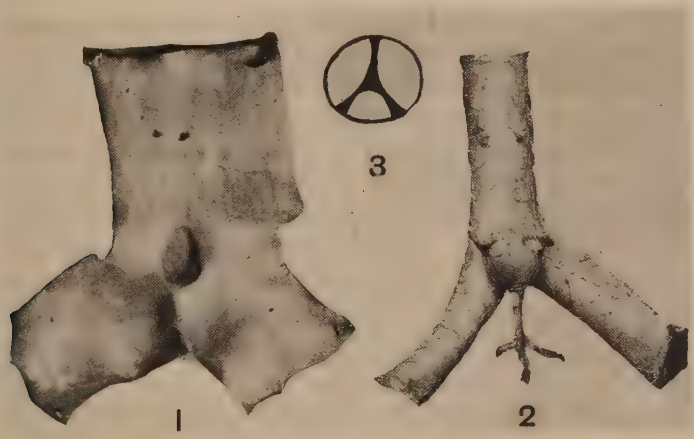
The specimen which forms the subject of figure 1 (a view from the side of the intima) and figure 2 (a view from the side of the adventitia—from the back) was taken from the body of a man, aged sixty, inhabitant of Odessa, of Russian nationality. Death was caused by nephro-pyelo-cystitis purulenta, which seems to have developed in connection with diabetes mellitus with which the patient was afflicted during life. The body was dissected on December 22, 1924, at the Medico-Legal Institute of the Odessa University, as death by poisoning was suspected. The suspicion, however, was disproved upon dissection.

The anomaly was disclosed when a section of the aorta was made laterally and longitudinally, in order to examine the intima.

Upon examination of the aorta from the side of the intima, an oval groove is at once seen in the posterior section of the bifurcation along the medial line, as if the lumen of the aorta continued beyond the bifurcation, though becoming very

much narrower. The common iliac arteries are separated from each other on the side of the lumen of the aorta by a medial ridge 8 mm. in length which does not extend to the posterior wall of the aorta, but bifurcates in its posterior part into two symmetrical branches which pass into the rounded edges of the groove described above.

In the lower portion of the abdominal aorta, in the ascending aorta, and in the adjoining portions of the common iliac arteries patches of adipose degeneration of the intima are found in the shape of pale yellowish spots from 1 to 7 mm. in



diameter. The intima of the groove does not show any of these changes. A test for transparency of the walls of the groove shows very little difference in this respect from the walls of the aorta.

An examination of the aorta from the side of the adventitia from behind (fig. 2) shows an oval swelling in the posterior portion of the bifurcation. The long axis of this swelling coincides with the longitudinal axis of the aorta. From the lower pole of this swelling the middle sacral artery emanates, widened at the beginning and bearing at a distance of 1 cm. from the beginning a pair of lateral branches. At the upper

pole of the swelling are seen the beginnings of the trunks of the fourth pair of lumbar arteries. The adventitia of the swelling as well as of the rest of the aorta exhibits no pathological deviations from normal.

The angle formed by the common iliac arteries equals 90°.

The two common iliac arteries are of approximately the same caliber. The seeming difference in thickness on figure 2 is due to the fact that the trunks in the course of treatment with preservatives were flattened in different planes: the right in the frontal plane and the left in the anteroposterior plane. Figure 2 represents the specimen as it appeared after the incisions were sewed together.

Figure 3 shows the place of bifurcation as it appears on the inside with the aorta sewed up. A tube of thin tin was introduced into the aorta, to prevent the walls of the trunk from shrinking, and the observed configuration was schematically drawn to approximately the natural size.

Some measurements of the anomalous formation are as follows:

A. Inside measurements

1. Length of opening leading into groove	13	mm.
2. Breadth of opening leading into groove	8.5	mm.
3. Length of medial ridge dividing iliac arteries	8	mm.
4. Diameter of orifice of middle sacral artery	1.5	mm.
5. Distance from orifice of sacral artery to fourth pair of lumbar arteries	11	mm.
6. Distance between fourth and third pairs of lumbar arteries	23	mm.
7. Distance between interior edges of orifices of fourth pair of lumbar arteries	5.5	mm.
8. Distance between interior edges of orifices of third pair of lumbar arteries	3	mm.

B. Outside measurements

1. Longitudinal diameter of swelling	15	mm.
2. Transversal diameter of swelling	11.5	mm.
3. Anteroposterior diameter of swelling	7.5	mm.

DISCUSSION

In seeking to account for the origin of this anomaly there are two theoretical possibilities: the anomaly may be regarded as a case of aneurysm developing on an originally

normal aorta or it may be placed in the category of irregularities of development.

We advocate the second interpretation for the following reasons: 1) A point against the hypothesis of the aneurysm is the exact symmetry of the groove and its regular and delicately fashioned edges, formed by the separation of the medial ridge of the bifurcation into two branches. 2) The absence, with the exception of the patches of adipose degeneration of the intima, of any other pathological changes in the wall of the aorta. As regards the patches of adipose degeneration, they are least marked in the neighborhood of the groove. 3) It is difficult to imagine conditions leading to the formation of an aneurysm in a place like the bifurcation where the flow of blood in the aorta is abruptly divided in two by a sharp ridge. 4) The angle of 90° between the two common iliac arteries points to arrested development.

This last circumstance must be especially emphasized. According to Houch ('03, p. 188), in early-stage embryos the iliac arteries form a right angle at the point where they originate from the aorta; but in an embryo of two months this angle is already equal to about 60°, which is the approximate average value in the adult. A series of measurements was made on bodies of adults and children of different ages, including newborn, and the angle formed by the two common iliac arteries was invariably found to be equal to 50°, and in no case to 90°.

This increase in the value of the angle could not be regarded as accidental to the procedure of sewing up the incisions. As a test, a normal aorta was taken with an angle of 50°, analogous incisions made and then sewed up: the angle was still equal to 50°. Besides, on figure 2 it is seen that with the specimen placed on a plane surface the axes of the two common iliac arteries intersect at right angles, while in the case of the specimen of a normal aorta this angle still remained 50°.

This evidence points to the view that in our case the process of convergence of the common iliac arteries was retarded,

with the uncommonly large angle of 90° as a result. At the same time, the cranial portion of the middle sacral artery did not become reduced.[1] This anomaly resembles those stages of intra-uterine development when, as in an 8-mm. embryo (Broman, '08), from one point of the aorta there arise three trunks of approximately the same caliber: two umbilical arteries and one primitive middle sacral or caudal artery which at that stage forms a direct prolongation of the aorta of a caliber steadily diminishing in the caudal direction. In our case, also, if the specimen is viewed through the lumen of the aorta (fig. 3) parts of three orifices are seen adjoining the ridge: on the sides and in front the common iliac arteries, and in the back the anomalous groove.

As already mentioned, Broman holds that the caudal end of the definitive aorta develops at the expense of fusion of the initial portions of the common iliac arteries. The case described here seems to confirm this theory. In fact, a necessary condition for such a combination is that the trunks should approach each other. In this case this process was retarded, with the result that the medial ridge of the bifurcation is insufficiently developed.

The retardation of the process of reduction of the cranial end of the middle sacral artery may be regarded as a secondary phenomenon occasioned by a difficulty in the flow of blood from the aorta into the common iliac arteries, in view of the angle of 90° between these latter. Under ordinary conditions, the flow of blood from the trunk of the aorta in entering either one of the common iliac arteries is deflected through an angle of from 25° to 30°, while in our case this angle is equal to 45°, and this might have caused local vortices in the blood. Having retarded the process of reduction of the cranial end of the middle sacral artery, these vortical currents in the blood must have tended to stretch the vascular wall in this region. This can be inferred from the fact that the distance between the two symmetrical trunks of the fourth pair of lumbar

[1] Either by diminution of its caliber or by becoming an integral part of the wall of the aorta.

arteries is somewhat greater than the distance between the trunks of the third pair (see table of inside measurements).

SUMMARY

On the body of a man, aged sixty, an oval groove about 1.5 cm. in length was discovered in the posterior portion of the bifurcation of the aorta. From this groove issued the middle sacral artery and the fourth pair of lumbar arteries. The angle included by the common iliac arteries was equal to 90° as against the normal 50° to 60°, pointing to retardation of development. On the strength of this, and also in view of the exact symmetry of the groove and the absence of pathological changes in the wall of the aorta (with the exception of patches of adipose degeneration of the intima of adjoining parts), this groove must be identified with the cranial section of the middle sacral artery which has not undergone the usual process of reduction. This anomaly seems to corroborate Broman's theory that the caudal end of the definitive aorta develops as a consequence of fusion of the initial portions of the common iliac arteries.

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